

## Scandalous Muddle in British Astronomy

THE letter in *Nature* from Professor Geoffrey Burbidge last week (239, 117; 1972) has brought into the open discontents about the management of British optical astronomy which have been simmering ever since the Second World War. Although the immediate cause of Burbidge's complaint is the resignation of Sir Fred Hoyle as director of the Institute of Theoretical Astronomy at Cambridge (see *Nature*, 236, 417; 1972), now irrevocable, the row which it will no doubt precipitate goes much deeper. And although there will now, in the polite tradition of the British, be a tendency to close ranks against an analysis of the mismanagement of astronomy which, for all its cogency, will be thought to be impolitely forthright, it is essential that the opportunity should now be seized for a thorough reappraisal of British policy in astronomy. At the same time, it should be acknowledged that the dormant chauvinism which has led to the record of muddle which Professor Burbidge has catalogued for astronomy is also malevolently at work elsewhere in the administration of British scientific research, pure and applied.

The events at Cambridge which led to Sir Fred Hoyle's resignation are in some ways a separate issue but not entirely irrelevant to the central theme of Professor Burbidge's complaint, for the university's grudging tolerance of Professor Hoyle and his theoretical institute typifies the way in which British institutions appear to be biased in favour of mediocrity. The original concept of the institute sprang from Professor Hoyle's enthusiasm for some facility at which modern computing machines could be applied to the solution of problems in astronomy—stellar structure, nucleogenesis and the like—but in the past few years it has also become a powerful centre of conceptual innovation, an intellectual centre of the highest quality. For this, whatever else may be said of him, Professor Hoyle was single-handedly responsible, yet there have always been people at Cambridge who have regarded the institute as an awkward cuckoo in the nest, with its independent and generous sources of finance and with its flair for recruiting astronomers from abroad as occasional fellows. The sensible scheme for integrating the institute with the University Observatories, agreed upon nearly two years ago, might have helped to take the edge off the academic jealousies, but it was exceedingly insensitive of the university to suppose that, under such a scheme, Professor Hoyle should not be given a powerful influence in the choice of a successor to Professor R. O. Redman, the retiring director of the observatories. (The most unhappy aspect of the whole affair is that Professor D. Lynden-Bell, the man eventually appointed, will no doubt be seriously embarrassed, which is why it cannot be said too often that he is a man of great distinction and high reputation—the issue is merely whether Professor Redman's successor should not have been an observational astronomer.) British institutions are fond of cutting innovators down to size in ways like

this, and the University of Cambridge is one of the more zealous among them.

Professor Burbidge's specific complaints about the other misjudgments which have now helped to determine the pattern of observational astronomy in Britain have too much of the ring of truth to be brushed aside. Whatever may be the value of the Isaac Newton telescope as a means of testing ancillary equipment, the experience of the past few years has amply justified earlier scepticism about the siting of a 90-inch reflector in the sea mists of Sussex. The unwillingness of British astronomers to collaborate with the European Southern Observatory project is reminiscent of Aesop's fable of the dog that saw the bone it was carrying reflected in the surface of a river—by sticking out for an institution that would afford them a substantial share of the observing time on a large southern telescope, British optical astronomers now find themselves in prospect of being shut out from a chance to use the European Observatory in Chile, due to be commissioned next year. Technically, the Anglo-Australian telescope has gone well, but there now remains an absurd ambiguity about the management of this instrument (see *Nature*, 239, 59; 1972) that should have been ironed out right at the beginning. And although there remain some doubts about the quality of the site in Australia selected for the telescope, it does seem plain that the Australian site was not investigated as carefully as that which has been selected in South Africa. Professor Burbidge's complaint that too much talent (and money) has gone on the development of equipment, and not enough on the making of observations, is harder to assess, for there may be something in the view that would-be astronomers blessed with poor seeing should devote themselves to techniques and not to observation—at best, however, this policy has been a gamble. Less divided management is an urgent need.

What is to be done? There is much to be said, where astronomy is concerned, for the creation of the national centre advocated by the Northern Hemisphere Review Committee and now, again, by Professor Burbidge. The Isaac Newton telescope could and should be moved to a site at which it could be used efficiently. The Royal Greenwich Observatory at Herstmonceux could and should become a provider of routine time-keeping observations, a smallish centre for theoretical and astrophysical research (preferably more closely linked with the University of Sussex) and a centre through which British interests in astronomical observatories abroad were administered. There is an urgent need to coordinate British ambitions in observational astronomy with those of other European scientists, to which end there should now be a modest if necessarily humble reapplication to join in the work of the European Southern Observatory. Above all, the Science Research Council (which is the chief influence in this field, and which spent close on £1.5 million on running observatories in the last financial year) should settle for a management procedure that endows the



enthusiasts with resources—and which saddles them with responsibility.

Why has this not been done before? The most accurate of all the charges that Professor Burbidge makes is that chauvinism is at some level to blame. For in the provision of tools for research, the British are notoriously unwilling to use equipment developed elsewhere and known to be reliable. Although there is much to admire in this desire to work things out from scratch, casting mirror blanks, designing clever mountings and novel ancillary equipment, the results are slow and cruelly frustrating for those who would make observations. A question of balance is involved, and many of the frustrations of the past few years, especially among young astronomers who have chosen to try their luck elsewhere, have arisen because the balance has been wrong. Devotees of homespun university telescopes, and of ancillary equipment that might improve their performance to the point at which people marvel that so much can be done in such unfavourable circumstances, forget that astronomy, like many other branches of experimental science (radio astronomy, for example), has become dependent on powerful telescopes, and that the nationality of the manufacturer's label is entirely irrelevant.

How far are the failings of the astronomer repeated elsewhere in British science? Ironically, it is only a week since the Science Research Council announced that it will abandon a plan to build a high flux reactor for neutron beam research and that it will instead collaborate with the French and German authorities now operating an appropriate reactor at the Laue Langevin institute at Grenoble. If this had been decided four years ago, time and ill-feeling would have been saved. Mercifully, where high energy accelerators are concerned, the Science Research Council has in principle decided to throw in its lot with CERN, and there are no longer hankerings after independent launching facilities for satellites. In other words, observational astronomy has all the appearances of the last bastion of an unreasonable sense of independence in pure research dependent on big machines. Unhappily, applied research is still coloured by too much of the belief that a British invention, late though it may be, is inherently better than something imported from abroad. Aircraft policy, the Concorde collaboration notwithstanding, is a touching proof of British faith in British turbine blades and British aerodynamics. British thermal reactors are likely, in due course, to be followed by British fast reactors. The trouble is that these policies of separatism are not merely an enormous waste of money and resources but an insufferable frustration for talent of the best kind—that of young and energetic people who want to get things done.

## Too Many Airlines

CIVIL air transport is potentially one of the most beneficial applications of technology, but is in danger of being mismanaged and misused by governments, with the connivance of the civil airlines. The symptoms of mismanagement have become pitifully apparent in the past few months, as the airlines have competed feverishly with each other to fill potentially empty seats by offering cheaper fares to a bewildering variety of potential passengers—students, retired people, people prepared to linger

en route and those prepared to book their tickets far in advance. At the same time, airlines fond of advertising their respectability find themselves being fined for breaches of the rules laid down by the international cartel, the International Air Transport Association, by which they communally organize their affairs for selling tickets at a discount and for providing travel agents and other sources of revenue with illicit inducements of various kinds. Yet the annual conference of IATA which opened this week at Torremolinos is likely to be again more preoccupied with cheap fares than with a reorganization of the basic fares on important routes such as that across the North Atlantic. And there is no end in prospect to the rash of bilateral disputes between governments about the supposed availability of too many seats on certain routes—the British and American governments, for example, have contended on behalf of national airlines that capacity must be restricted between London and Miami, while the government of Australia is resisting a demand by the government of Ceylon that the latter's airline should be allowed to fly to Sydney. The arbitrariness of these agreements is a measure of the wrongheaded economics of airline operations.

Two sets of issues need more public attention than they have been given. First, the notion that the amount of traffic between one country and another, or between one city and another within a country, should be determined either by bilateral negotiation between the appointed regulatory agencies or by a single agency (the Civil Aeronautics Board in the United States and the Air Transport Licensing Authority in the United Kingdom, for example) is innocent of considerations of efficiency. One result is that the governments which sponsor relatively inefficient airlines will argue for an unwarrantable restriction of traffic. Another is that airlines covered by such a bilateral agreement will devise timetables that send similar aircraft on identical routes at essentially the same times of the day, thus depriving potential passengers of as much flexibility as they could have. The second important group of economic problems stems from the constitution of IATA as a custodian of the profitability of the airlines. To begin with, it was a simple price-fixing cartel, eager to ensure that airlines should not compete vigorously on fares and even on the quality of service they provide. It has become, with the rash of concessionary fares in recent months, an instrument for making sure that airlines can fill their seats with people who might not otherwise travel without endangering the business they derive from those to whom travelling is a necessity.

The ideal remedy, not quickly attainable in the real world, is that the air transport industry should be able to make its own market unfettered by government negotiations and by the operation of a largely unregulated cartel among its members. The most that can be hoped for in the next few years is that governments and their airlines will admit a degree of competition sufficiently real to ensure that some at least of the airlines now in business will have occasionally to fear that inefficiency will lead to bankruptcy. As things are, this need never happen. Lilliputian Airlines will not go out of business so long as the government of Lilliput can trade landing rights with other airlines and raise the taxes needed to pay the necessary subsidies. One practical course of action would be to ask that bilateral agreements on landing rights



should have a modicum of flexibility—a confident airline covered by such an agreement might be allowed to increase its offering of seats by, say, 25 per cent above the norm agreed, provided that this was done at offpeak times. And where IATA is concerned, steps should be taken by governments acting as custodians for potential passengers, not as protectors of the airlines, to insist that fares on important routes such as the North Atlantic should quickly be reduced to a point which the efficient airlines consider would be economic. At present, fares tend to reflect what the inefficient airlines think they have to charge to keep in business.

## Olympics Must Change

ATHLETES and their sponsors are fond of saying that the quadriennial Olympic Games are a means of fostering international friendship, but there can hardly have been an occasion since the games were reinstituted in 1896 when the opposite seemed more likely to be true. It remains to be seen where the savage Israeli response to the savage terrorism in Munich last week will lead, but at best the hope of a permanent settlement in the Middle East has been delayed. But even without this flagrant violation of what the athletes fondly call the Olympic spirit, it had become shamefully apparent that the Munich games were an unhealthy outlet for base instincts of nationalism, chauvinism merging into xenophobia. For more than two weeks, newspapers all over the world have been full of exaggerated tales of the doings of national heroes at Munich, followed where appropriate by implausible explanations of their failure to perform as well as promised and sometimes by churchly reproofs. Countries such as the German Democratic Republic have been chidden for making competitive sport an instrument of international relations, but there is no evidence that comparable countries less successful at Munich were glad to have won a smaller share of the medals. Television cameras have been fixed lovingly on the flags of the countries at which their broadcasts were aimed whenever a national champion succeeded. The sordid spirit of nationalism was upheld by the decision of the International Olympic Committee that two black Americans should be banned forever from Olympic competition because they had not been sufficiently respectful when the United States flag was raised in their honour. What with the row about the admission of Rhodesia to the games and the bizarre declaration by the now mercifully retired president of the International Olympic Committee, Mr Avery Brundage, at the memorial ceremony for the dead Israelis last week that the pressure of black African countries which led to the withdrawal of the Rhodesian team was “naked political blackmail” comparable with terrorism in its gravity, it is plainly high time that the pattern of the Olympic Games should change.

What needs to be done is clear enough. When the Greeks held Olympic games, the rules were different and more civilized. For one thing, the competitors were individuals. For another, the competitions included rhetoric and drama as well as simple tests of brawn. Welsh eisteddfodau are a better contemporary model of the old festivals to Zeus than are the affairs which Mr Brundage

has been organizing since the Second World War. But the Olympic organizers must also be impressed, and made a little envious, by the way in which the competition between Fischer and Spassky at Reykjavik, in spite of all the preliminary hullabaloo (with chauvinistic trimmings, it is true), turned out to be an absorbing and an honorable contest of intellectual distinction.

The objective now should be to make the Olympic Games competitions between individuals designed so as to provide an opportunity for athletes to compete against each other as well as against objective yardsticks of performance culled from the record books. The idea that nations enter teams of competitors for the games should be, quite simply, abandoned. Team games should by the same test be outlawed—and if those who play basketball are anxious for there to be an international tournament, they should organize one for themselves. Boxing and wrestling should be dispensed with, not merely because they are unseemly but because knock-out competitions must of necessity involve luck at several levels. The fiction that those who compete in Olympic Games should be amateurs in the sense that they have never competed for money prizes or earned a salary from their sport should also be put aside—most simply by rewarding success at the Olympic Games with money prizes. The organizers of the Wimbledon tennis tournament have discovered, to their surprise, that this can be done without disaster. Whatever athletes may say, the world cannot allow these reforms to be neglected between now and 1976.

## 100 Years Ago



At the sitting of the French Academy of Sciences, on Sept. 9, a number of communications on the subject of the ravages of the *Phylloxera vastatrix* in the vineyards of France were read by M. Dumas, and referred to the “*Phylloxera* committee” of the Academy. It appears that the disease is making fearfully rapid advances in Provence, threatening the speedy entire destruction of the crop. In the department of Vaucluse it is also rapidly increasing; while in that of l’Hérault it is rather diminishing. All the correspondents agreed that when once a plant is attacked cure is hopeless, and that it is almost impossible to prevent the parasite spreading to neighbouring plants by any other means than complete submersion under water, though the application to the roots of a soil composed of sand, manure, and some insecticide, will delay it for some years. There is no doubt that the wingless insect migrates above ground from the diseased to the healthy plant, and is carried in great quantities by the wind. M. d’Armand, of Marseilles, demanded that a prize of 500,000 fr., or, if necessary, 1,000,000 fr., be offered by the State to any one who shall discover a means of arresting the disease. The pest has made great advances also in Portugal, especially in the neighbourhood of Porto, Villa Réal, Douro, and Santarem; and a Royal Commission has been appointed to investigate fully the causes of a disease which threatens the destruction of one of the most important branches of national wealth, and the best means of curing it.

From *Nature*, 6, 421, September 19, 1872.



## OLD WORLD

# British Association Out of the Doldrums?

from our Official Correspondent

*Leicester, September 9.*

AFTER one of the quietest and smallest annual meetings in recent years, the officers of the British Association for the Advancement of Science are nevertheless facing the future with a new optimism. The new constitution, financial security, the Royal Society's positive interest in the association's fate and the vast number of young people who invaded the Leicester meeting have all contributed to the new air of confidence in the BA's upper echelons.

For the first time in its 141 years, the association is to organize itself among students. At a meeting held in the dying moments of the Leicester gathering last Saturday, it was agreed that a student BA conference should be held, probably in January 1974, and that attempts should be made to set up a student organization in universities, polytechnics and colleges of education.

The British Association is not noted for the speed of its reaction, but its current attempt to tap student enthusiasm springs from the formation in 1969 of BAYS (the British Association Young Scientists) for school children. In four years BAYS has acquired 4,500 members in 46 branches around the country, throwing into relief the size of the membership of students in higher education which before the Leicester meeting numbered 147. Now, however, a student committee has been formed under the chairmanship of Ian Brimcombe, last year's BAYS chairman and a University of Oxford undergraduate, and the new constitution has provided the students with a seat on the association's council and therefore a say—as yet a small one—in policy.

Five hundred students and BAYS members turned up at Leicester—the largest number of young people at any BA meeting—and the BA, with an eye on the future and very conscious of the gap in its membership in the 20 to 40 age range, was mightily pleased to see them.

The new constitution also provides some hope for the future. The elephantine general committee, previously numbering 350 members, is to be cut to 59, with 36 of its members elected. The council, formerly 75 in number, is to be reduced to 22 members who will be elected by the general committee, but 12 of the 22 must come from the 36 elected members of the general commit-

tee. The BA hopes that this streamlined democracy will improve the image and the running of the organization, and, assuming the Privy Council approves the proposed changes, the new council and committee should be elected by the end of next year. Although strongly opposed in the past by some members, the changes this year passed the general committee unopposed and the lawyers, brought all the way to Leicester in case of trouble, returned to London unneeded.

The financial situation is now much easier (see *Nature*, 239, 59; 1972) and Sir Kingsley Dunham, the new president of the association, indicated that the Royal Society's new interest in the BA is not a passing one. The association is to receive £20,000 a year from the Department of Education and Science through the Royal Society, and Sir Kingsley, who is also Foreign Secretary of the Royal Society, explained that this support has sprung from the Royal Society's concern for the presentation of science to the public. "We (at the Royal Society) are absolutely clear that this kind of link is needed," Sir Kingsley said; "the BA was formed to do this."

But if the BA still has a role, what is it likely to be? The answer lies partly in the terms of reference of the Royal Society's grant to the association and partly in the way that the association is already moving. The grant is for "the popularization of science and the conducting of a responsible public debate on the social consequences of science", and this role was reflected in the symposia at the Leicester meeting on pollution, energy resources, women's liberation and genetic engineering. Further,

the association is sponsoring investigations into topics of current concern such as ethical problems of modern biology and medicine, and scientific collaboration in Europe, the details of which are intended, when published, to form the nucleus of "responsible public debate". "Science," Sir Kingsley said last week, "cannot be conducted in the way it once was in a vacuum totally disconnected from life", although he affirmed that the BA's annual meeting would still have plenty of basic science in its programme.

But while the BA was revolutionizing its structure and policy, the Leicester meeting was notably devoid of polemic and flag waving. The symposia on energy resources in this century and beyond took a sobering look at the trends in energy supply with predictions that supplies of natural crude oil would reach a peak in the 1990s and then steadily decline, with a consequent rise in price. Dr R. S. Pease, director of Culham Laboratory, and Dr T. N. Marsham of the UKAEA reactor group at Risley were cautiously optimistic that nuclear power, whether fusion or fission, can rise to meet the crisis, while Mr G. Armstrong, the National Coal Board's chief geologist, complained that the OECD countries (Britain included) show little sense of urgency about the possibility of an energy crisis in the 1980s.

The symposia on pollution took a dry look at the biological effects of pollution, with Dr Kenneth Mellanby warning that population control may prevent any marked improvement in our stock, ensuring that no superman is ever evolved, and possibly allowing pollution to do a great deal of damage unnoticed. With zero population growth approaching, an active pollution policy is vitally necessary, Dr Mellanby said. "If there are not pressures to eliminate those who are not well adapted to their environment, we may not recognize that we are all going round in a half-poisoned state, and environmental degradation could go unchecked."

Dr F. T. Blackaby, director of the National Institute of Economic and Social Research, provided one of the few politically orientated moments by warning that it may only be a matter of time before "some group or other successfully kidnaps a nuclear weapon and uses it for some kind of blackmail". Advocating the establishment of a peace research unit in Britain, Dr



Prof. Sir Kingsley Dunham

Blackaby suggested that the government should spend 1 per cent of its defence expenditure on the unit, which would amount to £2.5 million annually.

Mr Laurance Reed, MP, a member of the Select Committee on Science and Technology, urged that environmental control should not be carried out behind a veil of official secrecy. The River Pollution Acts and Alkali Acts "promote secrecy about industrial effluent and waste," Mr Reed said; "the argument that confidentiality is necessary to protect the commercial interests of the dischargers does not withstand examination." A file of analyses of all samples and other data should be kept, he added, its contents available for public inspection on the payment of a suitable fee. Mr Reed also urged the government to spend more money on pollution control, which at present costs between £4 million and £5 million a year. "This may be compared with a total of £600 million spent on all government research and development. By any standard this is small."

Taken as a whole, the Leicester programme was full rather than exciting, but ultimately the importance of this meeting—which 1,800 people attended compared with 2,150 at Swansea last year—lies more in the way that the association has looked to its future than in the way this particular BA went. And it is fitting that it is at Leicester that the BA finally became a little democratic. Simon de Montfort would have approved.

## MEDICINE

### Call for More Kidneys

HAEMODIALYSIS offers a patient with renal failure a better chance of survival than kidney transplantation, according to a report published this week. The rate of survival of patients who have received a kidney from a donor continues to increase but there is no such improvement among patients whose treatment involves haemodialysis.

The report comes from a joint committee set up by the Royal Colleges of Surgeons, Physicians, Obstetricians and Gynaecologists, and Pathologists, the Royal College of Physicians of Edinburgh, the Royal College of Physicians and Surgeons of Glasgow, the British Paediatric Association and the Renal Association, and provides the first review of the effectiveness of maintenance haemodialysis and transplantation in England and Wales.

The survey involved 1,120 patients (whose treatment was started between 1967 and 1970) from twenty-nine units in Britain which are equipped to carry out kidney transplants and haemodialysis. In spite of the imposing list of organizations represented on the committee, ten units did not bother to

reply and three sent in their returns too late to be included in the analysis.

The results show that about 60 per cent of those patients receiving one form of treatment or the other survived for more than three years. In the absence of treatment these patients would have a life expectancy of a few months at most.

Ninety per cent of the 728 patients undergoing haemodialysis survived for six months whereas 64 per cent survived for three years. Of the 365 patients who received a renal transplant—after varying periods of haemodialysis—87 per cent survived for six months and 59 per cent lived for three years. The survey shows that survival is dependent on age for this group, with patients under thirty-five having the greatest chances of survival.

The report points out, however, that in spite of the apparent similarity in the survival rates of the two methods of treatment, the survival rate of patients undergoing haemodialysis is significantly higher once allowance is made for the age of the patient. Also no patient can proceed to transplantation until he has undergone a successful period of haemodialysis, which also invalidates a direct comparison.

Since 1967 the number of patients undergoing maintenance haemodialysis in England and Wales has increased from nearly 200 to 1,128 (in September 1971). During this time the number receiving treatment at home has increased from less than fifty to 664, while hospitals during the same time have only had to cope with an increase from about 150 to 464. It is significant that the numbers treated in hospitals have been nearly constant since the middle of 1970.

To maintain an improvement in the treatment of patients suffering from kidney failure, the committee points out the need for a greater supply of kidneys. Between 1,300 and 2,200 people would benefit from maintenance haemodialysis every year in Britain, whereas 1,000 kidneys are needed each year for transplants. At present about 200 kidney transplants a year are carried out in Britain, a far cry from the number needed.

Donor organs must not be wasted and goodwill must be established between the transplant surgeons and coroners, according to the report. Transplantation should be considered first in an autopsy except in cases where this might interfere with the coroner's duty in establishing the cause of death.

A scheme, details of which will soon be announced by the Department of Health and Social Security, will go some way towards meeting the needs of the joint committee. The DHSS will probably institute a contracting-in scheme, in which people are to be

invited to participate by carrying a card which allows a surgeon in the event of the person's death to remove his organs. While this scheme by itself will probably not provide the necessary increase in the number of available kidneys, it is hoped that it will provide a nucleus from which a greater supply of kidneys will eventually become available.

## RESEARCH COUNCILS

### SRC Reshuffle

THE Science Research Council has streamlined its administrative staff following the retirements this summer of Mr C. Jolliffe and Dr W. L. Francis. Before the changes, the SRC had five directors, one each for the divisions of Administration, Science, Nuclear Physics, Engineering and Astronomy, Space and Radio, but now three directors will look after these five divisions.

Mr J. F. Hosie, previously director of the Astronomy, Space and Radio Division, is appointed director of Administration, and Dr A. W. Lines, previously director of the Engineering Division, now takes on responsibility for the Nuclear Physics Division as well. The third director is Mr M. O. Robins, who is taking charge of the Astronomy, Space and Radio Division as well as of 1970. The chances of survival of the Science Division. Mr Francis's position is filled by Mr Henry Walker, who was, until recently, director of Administration.

Mr M. O. Robins comes to the SRC from the Department of Trade and Industry where he was a Deputy Chief Scientific Officer in the Research Programmes Evaluation branch. Mr Robins is not new to the ways of the SRC, for he has been an assessor of the Science Research Council and its Science Board since March 1971.

Other changes include the appointment of Mr A. J. Eggington of Daresbury Nuclear Physics Laboratory to be head of the Engineering Division; Dr H. H. Atkinson of the Rutherford Laboratory, who has been seconded to the Cabinet Office, to be head of the Astronomy, Space and Radio Division; Mr I. A. Learmouth will move from the Astronomy Division to be head of the Nuclear Physics Division; Mrs J. O. Paton, at present Establishment Officer, will become head of the Science Division and Mr R. Edmonds is appointed Establishment Officer.

## SOVIET SCIENCE

### Venus-8 Data

from our Soviet Correspondent

THE first results to be published from the data returned by the Venus-8 probe, which landed on the illuminated



limb of Venus on July 22, 1972, present new information not only about the physical parameters of the atmosphere and its general chemical composition, studied by previous probes, but also about the illumination of the planet and the nature of the surface.

According to the official press statement (*Pravda*, September 10, 1972) the chief purpose of Venus-8 was the investigation of illumination. The photometer carried by the descent stage, which was sensitive to wide variations of luminosity, gave readings right down to the surface. A detailed quantitative analysis of the data is now being carried out, in order to determine the optical characteristics of the atmosphere. Preliminary estimates, however, indicate considerable atmospheric attenuation of the sunlight but "a definite fraction" does penetrate to the surface and on the surface "there exist considerable differences in illumination" between the day and night sides.

The day and night surface readings for pressure and temperature obtained for Venus-8 ( $470 \pm 8^\circ \text{C}$ ,  $90 \pm 1.5$  atmospheres) are very close to the readings of Venus-7 ( $475 \pm 20^\circ \text{C}$ ,  $90 \pm 15$  atmospheres) which soft-landed on the dark side on December 15, 1970, confirming the theoretical model of the atmosphere postulated on the basis of radio astronomy observation of the planet.

A gamma ray spectrometer, which had been calibrated against samples of Earth rock, revealed that the surface contained 4% potassium, 0.002% uranium and 0.00065% thorium, similar to terrestrial granite. Although these results give an insight of the geology of Venus, they are only of local significance, and no definite conclusions can be drawn about the evolution of the planet until further probes have investigated other regions of its surface.

In addition to experiments carried out on the surface and during descent, Venus-8 also provided more data on the upper atmosphere. Variations in the overload of the descent stage during aerodynamic braking were measured and because these variations depend on the atmospheric density they were used to estimate the density of the atmosphere above 35 km. In addition to physical measurements, Venus-8 supplemented the chemical data obtained from previous probes by studying the ammonia content of the cloud layers by a special reagent which changes from yellow to blue when it reacts with free ammonia. The colour change was recorded by photoresistances. At 46 km and 33 km,  $0.01\% - 0.1\%$  of ammonia was detected.

During the descent, the wind velocity was estimated by measuring lateral velocity of the capsule. Above 45 km, winds of  $50 \text{ m s}^{-1}$  were observed, decreasing to less than  $2 \text{ m s}^{-1}$  below 10–12 km. These data indicate a zonal

(latitude) wind directed from the terminator towards the day side—that is, the wind blows in the direction of the rotation of the planet.

#### AUSTRALIA

### Committee Assembled

THE government of Australia has at last settled the composition of the Advisory Committee on Science and Technology which is intended to give advice on the planning of a strategy for Australian research and development. The government's intention to have such a committee was first announced last April, and in the past few weeks the Prime Minister has been under pressure to say when the committee would be able to begin its work. One of the questions raised by the formation of the committee is the extent to which it will echo in its recommendations recent expressions of discontent with the Commonwealth Scientific and Industrial Research Organization, still the dominating force in Australian research and development. Industrialists have taken to complaining that the work of the CSIRO is not closely enough geared to the needs of industry (see *Nature*, **238**, 425; 1972).

### Chess Champion

A GREAT deal has been written in recent weeks about the progress of the Spassky–Fischer match both on and off the board. It is rare indeed that a chess match should arouse such a widespread following, but the quality of the games played (although always interesting) was probably lower than most people had hoped for. The drawn Botvinnik–Smyslov match of 1954 probably still has the best claim to be the championship match containing the highest standards of play since the Second World War.

Fischer's play was, nevertheless, entirely worthy of a world champion, and this was perhaps surprising in view of tensions that surrounded the start of the match. Spassky's play, on the other hand, lacked the authority and confidence exhibited in his earlier years (although he is still only 35). It seemed as though a form of morose fatalism had entered into his mental attitude towards the match and that this had adversely affected his play.

Yet at the start of the match a runaway Fischer victory seemed a fairly remote possibility. Fischer's incomprehensible error in the first game of allowing a bishop to be trapped, and his voluntary default in the second game, left Spassky with a two point lead. The initial phase of the match, however, started with the very next game

Among university scientists it is also held that some of the basic research supported by the CSIRO, in fields such as radio astronomy for example, would be better placed in universities. The eleven seats on the committee turn out to be shared by industrialists and academics in the ratio 6 to 5, which implies that some important arguments may already have been decided. The chairman is Mr Colin Lyne, director of the Australian Industrial Corporation and previously with Broken Hill Proprietary Company Limited. The university members of the committee are Professor A. J. Birch (Australian National University), Sir Rutherford Robertson (Australian National University and president of the Australian Academy of Science), Professor Colin Donald (Adelaide), Professor Robert Street (Monash) and Dr Bruce R. Williams (Vice-Chancellor, Sydney). The industrial members of the committee are Mr Peter Baillieu (King Ranch Pastoral Proprietary Company Limited), Mr Alan W. Hamper (ICI Australia Limited), Mr B. T. Loton (Broken Hill Proprietary Limited), Mr Russell T. Madigan (Hansley Holding Company Limited) and Mr John Wilson (Australian Paper Manufacturers Limited).

when Fischer displayed alarmingly good form and Spassky began to show the defensive approach that was to continue for many games to come. Fischer scored his first ever win against Spassky in this game, and he was to win no fewer than six further encounters with him before the match finally ended.

Spassky was generally poorly prepared for the openings that actually occurred in the match, and it was in this part of the game that Fischer seemed to have a noticeable psychological advantage. Spassky was reasonably happy when Fischer played an opening variation that he had frequently used in previous games (for example, the Sicilians of 4th and 11th games or the Ruy Lopez of the 16th game), but seemed ill at ease when confronted by a surprise opening choice by Fischer (for example, the Queen's Gambit of the 6th game or the Alekhine's Defence of the 13th game). Spassky tended to settle down only during the latter part of the match, but by this time Fischer could well afford to concede a series of draws.

It may be part of Fischer's technique to try to disturb his opponent's composure (either consciously or unconsciously) by somewhat dubious demands and complaints, but he probably does not really need to do so because he has shown himself to be the most gifted chess player in the world.—J. P.



## NEW WORLD

## Fast Reactor Breeds Controversy

by our Washington Correspondent

ALTHOUGH demonstration liquid metal fast breeder reactors (LMFBRs) are on the verge of starting up in Britain and the Soviet Union, and are well along the road in France and West Germany, the US Atomic Energy Commission is only now talking about arrangements for constructing the first commercial demonstration reactor in the United States. The reasons why the United States seems to be uncharacteristically lagging in this area of high technology are many and varied, but during the past two years the Atomic Energy Commission, at the personal behest of President Nixon, has been proceeding at breakneck speed towards the goal of commercial production of electricity from the LMFBR. And it has also run headlong into spirited opposition from environmentalist groups, and some scientists and economists.

In June last year, President Nixon sent an energy message to Congress in which he called the LMFBR "our best hope today for meeting the nation's demand for economical clean energy", and since then the Atomic Energy Commission has announced that a 350 MWe demonstration plant is to be built at Oak Ridge, Tennessee. If all goes according to the AEC's plan, the plant will be producing electricity by 1980. Last week, the arrangements by which this goal are to be met were reviewed by the Joint Committee on Atomic Energy. The committee nodded its grave head, asked a few searching questions, but showed no signs of modifying its unflinching commitment to the project. To give the appearance of even-handedness, however, the committee did give opponents of the project an opportunity to present their views, but precluded debate on their testimony by the simple but effective expedient of not asking them any questions.

The arrangements for constructing the demonstration plant, which are set out in a so-called memorandum of understanding adopted last month by the AEC and the utilities concerned, involve the Tennessee Valley Authority, which will provide the site and test and operate the plant, the Commonwealth Edison Corporation, which will supply top management personnel for the project, a company to be known as the Breeder Reactor Corporation and a second company called the Project Management Corporation. Essentially the Breeder Reactor Corporation (BRC), whose board of directors is drawn from the electricity companies

in the United States, will be responsible for collecting contributions from utility companies, and it will provide liaison between the project and the electricity industry. The Project Management Corporation (PMC), on the other hand, will be concerned with overseeing the development of the project and it will clearly have the chief executive authority.

One of the chief questions raised last week by the joint committee involved the financing of the project, a factor which is destined to become a bone of contention between critics of the LMFBR and the AEC. The issue is essentially this. The share of the financing from private industry has been set at \$250 million, while the Atomic Energy Commission will be required to foot the rest of the bill for construction of the reactor and five years of operation. The figure mentioned last week for the total bill is \$699 million. The arrangements discussed last week would commit the AEC to meeting cost overruns on the project, and the contention is that the Tennessee Valley Authority and Commonwealth Edison would have little incentive to stay within their budget if they knew that the taxpayer will foot the bill for cost overruns. After some questioning of AEC and electricity officials on this point, however, members of the committee seemed to be satisfied that the AEC would retain some control over the project, and that the arrangements are about the best that can be worked out to guard against cost overruns.

But the project's critics lack the joint committee's trust in the desire of the participants to keep within budget with little control from the AEC. Mr David Brower, President of Friends of the Earth, told the committee, for example, that "the AEC is trying to accept on the taxpayers' behalf an open-ended commitment to escalating costs and unlimited indemnities". He suggested that either public control over the project be increased or the public share of the funding be decreased "so that commercial participation in its support and in its administration are made commensurate". Moreover, on the same point, Mr Sam Love, Coordinator for Environmental Action, suggested to the committee that public representatives should be placed on the boards of the BRC and the PMC. The joint committee's refusal to discuss these two statements gave little indication of the members' feelings about them.

Financial control over the project, although a symbolic as well as an

important point of principle as far as opponents of the project are concerned, is by comparison one of the minor criticisms that have been raised so far. More far-reaching are criticisms directed towards the safety of the LMFBR, its economics and the fact that it is receiving priority funding while other sources of energy such as solar power, geothermal power and controlled thermonuclear fusion are receiving inadequate attention.

The project's critics have charged, for example, that because fast breeder reactors produce large quantities of plutonium, which is the chief constituent of a simple atomic weapon such as the bombs dropped on Hiroshima and Nagasaki, the potential for clandestine diversion to other countries is greatly increased. Moreover, since plutonium is an extremely long lived isotope and is highly toxic, an accident in an LMFBR would be even more devastating than an accident in a plant fuelled by enriched uranium alone. Critics of the project have also pointed out that if energy production is based on the fast breeder reactor (the AEC estimates that more than a hundred may be operating by the year 2000), enormous problems of transporting plutonium will arise, and they claim that this question has not been properly addressed by the AEC.

These points were all raised in a statement signed by 31 scientists in April this year, who asked for a moratorium on the construction of an LMFBR. The signatories include Linus Pauling, Barry Commoner, John Edsall, James Watson and George Wald. Moreover, the Scientists' Institute for Public Information filed suit in the District Court claiming that the AEC's environmental impact statement for the demonstration project was inadequate and should have a discussion of the impact of the complete programme which will develop if the demonstration plant proves to be successful. The suit has been turned down by the court but it is being appealed.

There seems therefore to be little chance that the project will now be stopped by opposition from environmentalists or other groups. The Administration is firmly committed to the LMFBR, the money has already been authorized by the Joint Committee on Atomic Energy, and the committee has consistently reaffirmed its support for the breeder reactor concept. The chief obstacles in its path are now technical and it is unlikely that American technology will falter.

USSR

## Academy Weighs Protest

by our Washington Correspondent

THE National Academy of Sciences is considering lodging a protest with the government of the USSR over the persecution of Professor B. G. Levich. An eminent electrochemist, Levich was recently dismissed from his position as head of the Institute of Electrochemistry, deprived of his professorship at the University of Moscow and banned from taking part in the International Biophysical Congress in Moscow last month, presumably because he had applied for a permit to emigrate to Israel (see *Nature*, **238**, 365; 1972).

The form of the Academy's protest—or, indeed, if there is to be one—has not yet been finally decided. But the foreign secretary of the academy, Dr Harrison Brown, has been meeting with officials of learned societies in Europe, including the Royal Society, during the past two weeks to sound out the prospects for lodging a combined protest. The decision to explore such a possibility was taken at an NAS council meeting last month, but whether or not the protest should be made public was not decided. The issue was, however, resolved for the academy last week when news of the possible protest appeared in the press.

If the protest is made, it will probably steer clear of condemning the general policies of the USSR concerning emigration to Israel, but it will concentrate on the harassment of scientists, particularly the situation in which an eminent and productive scientist such as Levich is prevented from working for overtly political reasons. Similar protests from the academy have in the past been made on an individual basis, and they have not been made public.

In the meantime, the case of another Soviet scientist who has been persecuted for similar reasons seems to have taken a turn for the worse. Dr Alexander Ya. Lerner, who was summarily dismissed nearly a year ago from his professional positions in the Moscow Institute of Control Sciences and from his professorship at the University of Moscow after he had applied for permission to emigrate to Israel (see *Nature*, **235**, 121; 1972), has also been prevented from accepting an invitation to give a series of lectures in Italy and he was recently refused permission to attend the Congress of the International Federation of Automatic Control (IFAC) in Paris.

Although, in view of the previous harassment of Professor Lerner, it is not surprising that he was denied permission to travel outside the Soviet Union to international conferences, a particularly unsavoury aspect of the affair is what seems to be a blatant attempt by the government of the USSR

to interfere with the internal affairs of IFAC. Lerner is vice-chairman of the IFAC Committee on Applications, but Dr V. A. Trapeznikov, his former director at the Institute of Control Sciences, has demanded that he be removed from this position. The demand has been refused.

Lerner was refused permission to attend the IFAC Congress because of "insufficient foreign currency", in spite of the fact that all his expenses would have been paid by IFAC. Moreover, to add insult to injury, Trapeznikov asked that the paper Lerner was intending to deliver at the congress be removed from the programme because it was found by his co-author to be based on errors. The programme committee screened the paper twice, however, and denied the request.

## CARDIOVASCULAR DISEASES

### More Funds for Research

by our Washington Correspondent

CONGRESS last week finally passed and sent to President Nixon a bill calling for massive increases in expenditure on research into diseases of the heart, lungs and blood vessels. Originally sponsored in the House of Representatives by Paul G. Rogers of Florida and in the Senate by Senator Edward M. Kennedy of Massachusetts, the bill launches a crusade against diseases of the cardiovascular system similar to the crusade against cancer. It calls for expenditures of \$1,380 million over the next three years, mostly administered by the director of the National Heart and Lung Institute, part of the National Institutes of Health.

Apart from increasing the budget of the National Heart and Lung Institute, the bill also calls for the establishment of fifteen new centres for basic and clinical research into diagnosis, prevention and treatment of cardiovascular diseases, together with fifteen new research centres similarly devoted to chronic diseases of the lungs.

If President Nixon signs the bill—and it is unlikely that he will veto it in election year, even though it calls for more expenditure than he would like—the money entailed would still have to be appropriated by the appropriations committees. The committees are now in the throes of drawing up fresh appropriations for the National Institutes of Health after President Nixon vetoed the appropriations for the Department of Health, Labor and Welfare for the present fiscal year, and they are under orders to trim down their original bill considerably. That bill, however, contained some \$75 million less for the National Heart and Lung Institute than the bill passed last week requested. There is therefore little chance that the sponsors will get all they ask for.

## AUTOMOBILES

### GM Goes Rotary

by our Washington Correspondent

DETROIT has been going through a tough time lately. Not only must automobile manufacturers produce cars that meet stringent air pollution control standards in 1975, but last week the Administration turned down their request for a price increase on 1973 models. With all the concern over air pollution control devices and the industry's claims of financial hardships, this would seem a bad time for major innovations in the motor industry. But, on the very day that its request for a price increase was turned down, General Motors announced that it would offer rotary engines in its popular Chevrolet Vega cars about two years from now.

Although GM would then be the only American motor manufacturer to produce cars with the Wankel engine, its decision to go rotary was prompted to some extent by the success on the West Coast of the Japanese Mazda. Another factor is the rush to meet the 1975 air pollution requirements, for although the Wankel engine is not inherently clean, it may be more adaptable to the catalytic converters on which the motor industry now seems to be pinning its hopes. One problem with the catalytic converters is that they are bulky, which makes them difficult to reconcile with the industry's obsession with body styling, but the Wankel engine is only about two-thirds the size of a conventional engine with comparable power, so that the converters can be fitted without sacrificing advertising appeal.

Named after its German inventor, Felix Wankel, the rotary engine runs on a mixture of air and gasoline, but it differs from the conventional automobile engine chiefly in the combustion chamber. Instead of pistons, the central part of the engine consists of three triangular rotors which rotate in an elliptical chamber to compress the fuel mixture, which is ignited by spark plugs.

The engine contains many fewer parts than the conventional piston engine, and it is reputed to be quieter, but it has the disadvantage of being much more costly to produce. Although GM spokesmen are reluctant to discuss costs at present, they said last week that the company is attempting to improve the durability of the inside wall of the chamber and the apex of the triangular rotors which are in contact with it, and which take the chief frictional wear. At least one major cost of the engine to GM is also the licence fee that is being paid to the West German patent holders, for worldwide rights. GM is reported to be paying Audi/NSU \$50 million for the rights.



## NEWS AND VIEWS

# Lasers and Controlled Thermonuclear Fusion

RESEARCH into controlled thermonuclear fusion seems to be taking on a new lease of life. On the one hand, the relatively long plasma confinement times that have been measured in the present generation of "conventional" toroidal magnetic devices are widely regarded as encouraging; coupled with good scientific progress in correlating these results with theory, there is an increasing interest in the environmental benefits which might accrue from power generation by controlled fusion. On the other hand, several new approaches to the fusion problem have also been receiving publicity. The article on page 139 of this issue of *Nature* discusses one such approach—the inertial confinement of a plasma ignited by a laser.

The idea of using a laser to heat matter to very high temperatures is probably as old as the laser itself, for the extraordinarily high brightness of laser light has been well recognized since Maiman's first demonstration of lasing action in ruby in 1960. The significance of the contribution made by Nuckolls and his colleagues, working at the Livermore Laboratory of the University of California, may therefore be best appreciated by outlining developments which have been reported in the open literature and at unclassified conferences in the past three or four years.

In 1968 Academician N. G. Basov announced the first observation of neutrons generated in plasma produced by a laser; within a year similar experiments were reported from France and the United States. For such heating experiments, it is necessary to deposit the laser energy in a time shorter than the hydrodynamic expansion time of the plasma; this "inertial time" is characteristically a few nanoseconds or less. Laser technology has evolved steadily since these initial experiments, and individual laser pulses with high spatial coherence, a pulse duration of a few nanoseconds, and energies approaching  $10^3$  J have now been reliably produced in the laboratory. In spite of these interesting experimental developments, published estimates of the laser energy required to heat an isolated, deuterium-tritium pellet to conditions of marginal interest in the context of a reactor, at (approximately) solid state densities, were extremely high (of the order  $10^8$  to  $10^9$  J). The calculations implied therefore that very significant advances in laser technology would be needed to produce plasmas appropriate to a reactor.

Furthermore, assuming net laser heating efficiencies of about 20 per cent, the pulsed thermonuclear output of a system producing power could well have an energy equivalent of many tons of TNT. Although suitably designed cavities might contain such explosions, the cost of replacing damaged or disposable components would need to be exceedingly low for the system to be of economic interest.

It was widely recognized that it might be possible to reduce these onerous energy requirements by compressing the target, or alternatively by tamping its subsequent expansion. The first possibility is particularly attractive in spherically symmetric geometries; assuming a target radius (and hence an inertial confinement time) sufficiently large to satisfy the Lawson criterion for thermonuclear burn, the energy which must be invested in

heating the target plasma should decrease inversely as the square of its density.

How might the target be compressed without heating it prematurely, what degree of compression is possible, and what thermonuclear gain might be expected? Detailed answers to these questions have proved to require very significant computational effort, in fields which were traditionally related to military programmes. Thus, the funding of research into laser fusion in the United States has come primarily from the Division of Military Application of the US Atomic Energy Commission.

The decision to publish some of the American plasma work with lasers was announced by Professor Teller of the University of California at the Seventh International Quantum Electronics Conference in Montreal in May this year. Commenting on this decision, Professor Teller said he hoped that its impact would be comparable with that of the talk given by Academician I. V. Kurchatov at Harwell in 1956, which had led to wide international collaboration on the physics of magnetic confinement. The article by Nuckolls and his colleagues describes that part of the USAEC programme which has so far been declassified, and which was presented in post-deadline talks at the Montreal conference.

In a statement to the Joint (Congressional) Committee on Atomic Energy on November 10, 1971, Dr R. L. Hirsch of the US Atomic Energy Commission reported that a private organization, KMS Fusion Inc., is also working under a no-fund (monitoring) contract with the AEC on research into laser fusion. Dr K. A. Breuckner described some of the theoretical work completed at KMS in unpublished talks given at the Universities of Michigan and California (San Diego) in April and May this year. Significant aspects of the KMS programme are said to be covered in classified patent applications currently being reviewed by the US Patent Office. It seems very probable that similar calculations have been pursued by the other nuclear powers.

The Livermore group does not claim that reactor feasibility has been definitively demonstrated by its calculations; indeed, a wide range of interesting physical problems is raised by the work. At the laser intensities discussed in the article, interactions with the (inhomogeneous) plasma are highly complex; non-linear plasma physics becomes of great importance, and at the longer wavelengths relativistic effects can also occur. These considerations affect, for example, the non-linear absorption of laser light in the outer atmosphere surrounding the pellet, back scattering of the incident light, unwanted preheating of the core by high energy (non-thermal) electrons, self-focusing, and penetration of radiation beyond the critical density. These wavelength-sensitive factors complicate the choice of lasers for definitive experiments or studies of reactor feasibility.

Similarly, complete spherical symmetry in the direction and polarization of the incident radiation is necessary to avoid thermoelectric magnetic field effects, which have been omitted from the calculations. Nevertheless, the principal computational effort described by the Livermore group has pointed to the tremendous compressions

(to densities approximately  $10^4$  greater than solid state densities) which may become possible with the development of suitably tailored laser pulses. At these very high densities heating of the target by fusion products (both  $\alpha$  particles and 14 MeV neutrons) can occur, and significant thermonuclear gains can be expected at relatively low laser energies. If hopes raised by these first predictions are fulfilled, several useful and important differences between the scaling of reactor systems depending on magnetic and inertial confinement are anticipated.

Expenditure by the USAEC on plasma work with lasers is reported to be about \$20 million a year at present, while the very strong experimental programmes under Academicians Basov and Prokhorov at the Lebedev Institute in Moscow must involve a comparable effort.

In Britain, the Culham Laboratory of the Atomic Energy Authority first published work on the laser heating of isolated hydrogen pellets in 1967; plasmas produced by lasers have subsequently been used for filling magnetic traps. Important contributions to the laser-plasma field have also been made by several of the universities, notably Belfast, Essex and Hull.—I. J. S.

## Cyg X-3 Blows Up

RADIO astronomers have had a busy time these past two weeks. On Saturday, September 2, at 2200 UT observers at the Algonquin Radio Observatory in Ontario recorded that the X-ray source Cygnus X-3 had suddenly become one of the brightest sources in the radio sky at their observing wavelength of 2.8 cm, an astonishing discovery considering that the first detection of radio emission from Cyg X-3 was announced only two months ago.

Since the Canadian discovery communications between observatories have been at a high pitch. By early Sunday morning the outburst from Cyg X-3 had been confirmed at seven wavelengths from 1.9 cm to 21 cm following an immediate alert of other radio observatories by the observers in Canada. In France observers at the Radio Observatory of Nancay have estimated the distance of Cyg X-3 by observing that the 21 cm hydrogen line emission from Cyg X-3 shows absorption due to the spiral arm at 8 kpc, but no absorption that could be due to the 11 kpc arm (1 kpc = 3,260 light years). Cyg X-3 must therefore be between 8 kpc and 11 kpc.

It is, of course, early days yet, but it is clear that an unprecedented eruption of some kind has taken place on Cyg X-3. The more optimistic will point out that we are overdue for another supernova—the last to be recorded in our Galaxy was a good three hundred years ago. But so far there have been no reports of the optical detection of Cyg X-3—possibly not surprising because that part of the Cygnus constellation is heavily obscured.

The signal recorded at Algonquin was 22 flux units (1 flux unit =  $10^{-26}$  W m $^{-2}$  Hz $^{-1}$ ). Before then the strongest signal from Cyg X-3 at that wavelength had been about 0.5 flux units, and the signal had often been less than that. But it was clear from observations made earlier this year and last year at the Westerbork observatory in Holland—where the radio emission was first detected—and at the National Radio Astronomy Observatory in West Virginia that the signal from Cyg X-3 can be extremely variable on a time scale of hours and that it could therefore be classed as an unusual source.

There seems to be no evidence so far that Cyg X-3 is behaving like a pulsar.—By our Astronomy Correspondent.

## Dapsone and Leprosy

THE causal agent of leprosy, *Mycobacterium leprae*, cannot be cultured on cell-free bacterial media. The discovery that leprosy bacilli could survive and multiply in the foot-pads of normal mice, and especially in those rendered immunologically deficient by thymectomy and whole-body irradiation, has revolutionized research into the pathogenesis and treatment of the disease. Of the drugs that have been used in the treatment of leprosy, dapsone (4,4-diaminodiphenyl sulphone, DDS) is still the standard agent, and its minimal inhibitory concentration can be estimated *in vivo* by measuring its concentration in the serum of mice fed with the minimal effective dose required to inhibit bacterial multiplication in the foot-pads. It has recently been found that dapsone is converted in man to monoacetyldapsone (MADDS) by the same enzyme system responsible for acetylating isoniazid and sulphamethazine. Indeed, the acetylation of isoniazid is important in the inactivation of the drug, and those subjects who are completely deficient in the enzyme may develop polyneuritis because of high levels of circulating isoniazid. But, unlike acetylisoniazid and acetylsulphamethazine, MADDS is rapidly deacetylated in man. The half-life of dapsone seems to be unrelated to its speed of acetylation.

In the article by Ellard *et al.* on page 159 of this week's issue of *Nature*, an attempt is made to determine whether the rate of acetylation of dapsone is of prognostic importance in the treatment of leprosy. It has been found that patients of low acetylator phenotype can respond favourably to isoniazid when on suboptimal regimes, and Ellard and his colleagues have made use of this observation to test the acetylation status of two groups of patients with leprosy. One group had received a hundredth of the conventional dose of dapsone in a pilot chemical trial, and yet had responded initially as well as those on the usual dose. The other group consisted of patients who had relapsed after many years of treatment with conventional doses; the relapse was due to the emergence of strains of leprosy bacillus resistant to dapsone.

The acetylator phenotype of the patients was assessed in terms of their capacity to acetylate isoniazid. The level of isoniazid in the plasma eight hours after its oral administration and the amount of acetylisoniazid excreted in the urine during the first two hours were the measurements used. In the first group of patients there were some rapid acetylators, and these responded to treatment during the first months as satisfactorily as did the slow acetylators. In the second group there were also some rapid acetylators, but in both the slow and the rapid acetylators, bacilli were able to multiply in the foot-pads of mice given an oral dose of dapsone equivalent to the conventional dose in man.

Ellard and his colleagues induce from these results that the rate of acetylation of dapsone is not a significant prognostic factor in the treatment of leprosy. It may be that MADDS has intrinsic antileprotic activity, but, as it is rapidly deacetylated to dapsone both in man and mouse, its therapeutic efficiency cannot at present be established.—M. I.



## SEISMOLOGY

**Nocturnal Earthquakes**

from our Geomagnetism Correspondent

THE suggestion that seismic activity fluctuates with the seasons or the time of day has been made on numerous occasions in the past; but as Bullen (*Introduction to the Theory of Seismology*, Cambridge University Press; 1963) pointed out some years ago, claims for such periodicities have never been substantiated. Not, that is, until last year when Shimshoni (*Geophys. J.*, **24**, 97; 1971) produced evidence which seemed to show that seismic activity is significantly higher during the night than during the day. At first sight such a correlation appears inherently unlikely, except that it is never possible to ignore completely the idea of trigger forces. In other words, although there can be no suggestion that minor forces, such as, for example, temperature effects, tidal effects and abnormal changes in barometric pressure, produce the elastic strain which is ultimately released in earthquakes, it is just conceivable that minor forces act as the "last straw" (Bullen) leading to the actual release of strain.

But whatever the cause may be, a correlation is a correlation; and that is just what Shimshoni seemed to have from a statistical analysis of the 15,325 seismic events reported by the US National Oceanic and Atmospheric Administration for 1968 to 1970. If seismic events occur randomly with local time throughout the twenty-four hour period, the number of events within each hourly period over the three years should be 638.5 (that is, 15,325/24). In practice, however,  $\chi^2$  for the randomness hypothesis turned out to be 80.1137, the probability of obtaining a figure as high as this for a truly random process being less than  $10^{-7}$ . Shimshoni thus concluded that seismic events do not occur randomly throughout the twenty-four hours, and went on to show that within the daily cycle there is a peak of seismic activity around midnight and a minimum around noon.

Three authors or groups of authors have now descended upon Shimshoni to point out that, although there may indeed be a correlation of the sort he demonstrates, it represents a data deficiency rather than a real variation in seismicity. Davies (*Geophys. J.*, **28**, 305; 1972), for example, notes that the number of earthquakes recorded over any given period of time will reflect not only the number of earthquakes actually occurring over that period, but also the detection capability of the recording network, and that it is the latter which varies between night and day.

To see how this effect works, he considers an earthquake occurring on the equator at the equinox. The location of

such an event will be determined from observations made within epicentral distances of  $100^\circ$ ; and so if the shock occurs at midday it will be observed (P waves) at most of the relevant stations in daylight, and if it occurs at midnight it will mostly be observed in darkness. Seismic background noise—most of it probably local cultural noise—correlates strongly with light and dark, however, the detection threshold at many stations being significantly higher during the day. According to Davies, it is possible to observe at many stations a dawn noise level rise of 10 to 20 db.

The result of all this is that more of the earthquakes occurring at night will actually be detected—a circumstance which is bound to bias the data in any published list of earthquakes. On the other hand, this situation will not obtain for events occurring far from the equator. At high latitudes, P waves from midnight earthquakes will be observed at many stations during daylight. Under these circumstances no day/night correlation would be expected; nor is it found. Events occurring at middle and lower latitude will, however, be sufficient to give an overall bias.

Flinn *et al.* (*Geophys. J.*, **28**, 307; 1972) raise the same basic objection as Davies, but they also present data to make explicit the point that it is the detection rate of the low magnitude earthquakes that will increase as the detection threshold lowers at night. Moreover, this will have a particularly significant effect in giving the Shimshoni correlation simply because most events are of low magnitude anyway (only 4 per cent have body wave magnitudes greater than 5.5). In a very satisfying series of graphs of earthquake frequency against the time of occurrence, Flinn and his colleagues are able to show that the

diurnal effect is definitely not present for events in the body wave magnitude range 5.5 to 6.5, is possibly present in the range 4.5 to 5.5, and is certainly present below 4.5.

Knopoff and Gardner (*Geophys. J.*, **28**, 311; 1972) again raise the question of bias introduced by the higher daytime noise, but they also add two other possible sources of bias. First, they claim that there is bound to be a bias against reporting small shocks because of a "finite and variable density of mesh of seismic stations". In addition, they propose a bias caused by aftershocks. The argument here seems to be that if one of the relatively few earthquakes of magnitude 8 or higher happens to occur at night the large number of associated aftershocks will give a false impression of nocturnal seismicity. Knopoff and Gardner then go on to describe an elaborate technique for the identification of aftershocks and their elimination from the record. When the three sources of bias are removed from the original record (actually for the period March 24, 1963, to December 31, 1968, rather than 1968 to 1970), no day/night correlation is evident. The conclusion then is that Shimshoni's correlation must be produced by one or more of the three biases.

As Shimshoni (*Geophys. J.*, **28**, 315; 1972) points out in his reply to the critics, the elimination of aftershocks by Knopoff and Gardner is rather "drastic", not least because the process will also remove many events not related to the principal shock. On the other hand, he accepts the basic point about variation in detection capability. But this is not necessarily the last word on the matter. The question still remains as to whether the detection threshold effect can explain all of the day/night correlation.

**A New DNA Base**

IN *Nature New Biology* next Wednesday (September 20) Marmur and his several colleagues report the identification of a new DNA pyrimidine 5-(4',5'-dihydroxypentyl)-uracil, or DHPU, which is present in the altogether unusual DNA of bacteriophage SP-15, a generalized transducing phage of *B. subtilis* and *B. licheniformis*.

DNA from SP-15 phages has an anomalously low melting temperature ( $61.5^\circ\text{C}$ ) but a very high buoyant density ( $1.761\text{ g cm}^{-3}$ ). From past experience with the DNAs of other phages Marmur and his colleagues suspected that these unusual properties of SP-15 DNA might well result from the presence of modified bases, and their suspicions proved to be well founded. After SP-15 DNA is hydrolysed with 90 per cent formic acid, five rather than four bases can be separated by thin layer chromatography. The

new fifth base partially replaces thymine to the extent that it comprises 12 moles per cent of all bases. Marmur's group and collaborators isolated this base, DHPU, on a preparative scale and determined its structure by spectroscopic and nuclear magnetic resonance measurements.

The substitution of thymine by DHPU probably accounts for the low melting temperature of SP-15 DNA, but it does not account in any obvious way for the very high buoyant density of this DNA. That apparently results from a compound which is covalently linked to both strands of the DNA and reacts as a pentose. This pentose compound may be in the phosphodiester backbone of the molecule, but it might rather occur attached to the side chain of DHPU. Finally SP-15 has one other unusual feature: it includes covalently bound glucose residues.

## PLANT BREEDING

**Parasexual Tobacco**

by our Botany Correspondent

A FURTHER step towards a means of producing hybrid plants without recourse to sexual reproduction has been reported from Brookhaven National Laboratory in New York. Three members of the department of biology have grown viable plants from fused cells of two species of tobacco, *Nicotiana glauca* and *N. langsdorffii* (P. S. Carlson, H. H. Smith and R. D. Dearing, *Proc. US Nat. Acad. Sci.*, **69**, 2292; 1972). Thus they have combined the previous Japanese and British achievements, when isolated protoplasts—the naked contents of plant cells—were induced, on the one hand, to grow into whole plants, and, on the other hand, to fuse with protoplasts from another plant without further development.

The Brookhaven group used similar techniques to obtain and fuse protoplasts from the two species of *Nicotiana*. Strips of epidermis from young leaves of each species were incubated in an enzyme mixture containing cellulase, macerozyme and sucrose to dissolve away the cell walls. Protoplasts were collected by low speed centrifugation, and then the two species were suspended in sodium nitrate solution, which had previously been shown to induce fusion.

After thirty minutes a pellet was spun down, resuspended and plated on a nutritive medium. At this stage Dr Carlson and his colleagues had a mixture of fused hybrid protoplasts and unfused parental protoplasts of both species. The methods used to select out the hybrid cells were based on knowledge of the biochemical characteristics of the hybrid *N. glauca* × *N. langsdorffii* which had already been produced by orthodox sexual means. Regeneration media were used on which only the cells containing the genetic information of both parents would be able to grow.

Out of more than  $10^7$  protoplasts of each parental species, thirty-three hybrid cells developed into calli which produced rudimentary shoots and leaves but no roots. Further development was obtained by grafting the regenerated shoots onto cut stems of young *N. glauca* plants.

Morphological and biochemical characteristics of the resulting plants, and the flowers and seeds they have now produced, compared exactly with those of the sexual hybrid between the two tobaccos. This confirmed that the parasexual methods used had yielded true hybrids. Of course the next goal in this work is to develop hybrid plants from parents that cannot be mated in the conventional manner, but nevertheless have characters that plant breeders would like to see combined. At present,

however, there are technical problems to be overcome. The exact conditions have to be found for the regeneration of the fused protoplasts, and there is also the problem of distinguishing hybrid from parental tissue after growth has been induced. It will be interesting to see the next development in this branch of the new botany.

## HISTONES

***In vitro* and *in vivo***

from our Cell Biology Correspondent

DURING the past several years a class of RNA molecules which sediment at 7-9S has, with increasing confidence, been claimed to contain messenger RNAs for histone proteins. These putative histone mRNAs have several properties that distinguish them from other putative and identified messengers. For example, they appear to lack the 3' terminal tracts of polyadenylic acid present in many other messengers; they are transported from the nucleus to the cytoplasm more rapidly than most other RNAs; they appear in small cytoplasmic polysomes only when the cell is replicating its DNA, and they seem to be specified, at least in sea urchin cells, by reiterated and clustered DNA sequences. But, of course, none of these properties of putative histone mRNAs prove that these molecules actually specify histones, which explains why the report of Jacobs-Lorena, Baglioni and Borum (*Proc. US Nat. Acad. Sci.*, **69**, 2095; 1972) will be greeted with signs of relief in many quarters. For they have shown quite conclusively that the 7-9S putative histone mRNAs isolated from the polysomes of populations of synchronized HeLa cells in S phase are indeed trans-

lated in a cell-free system obtained from mouse ascites tumour cells to yield polypeptides which include all five classes of human histones.

The 7-9S polysomal RNAs extracted from HeLa cells stimulated protein synthesis in the ascites cell-free system from four- to eleven-fold; the histones were identified amongst the products of translation by electrophoresis in SDS and acidic polyacrylamide gels, and by comparing tryptic peptides obtained from the polypeptides made *in vitro* with those obtained from authentic human histones. Whether or not the 7-9S RNA, as prepared by Jacobs-Lorena *et al.*, contains messengers other than those for histones, and whether or not RNAs other than messengers are present, remains to be seen. The fact that the 7-9S RNA from HeLa cells failed to stimulate appreciably incorporation of tryptophan, an amino-acid absent from histones, suggests that the histone mRNA was not extensively contaminated with mRNAs for other proteins, but the possibility that it contained messengers for a non-histone protein deficient in tryptophan cannot be excluded. The fact that the histone mRNA preparations had less messenger activity in the cell-free system than preparations of haemoglobin mRNA may mean that RNAs lacking messenger activity were present as contaminants, but such differences in messenger activity, which is assayed *in vitro*, can be explained in several other ways; for example, histone messenger may be more labile than haemoglobin messenger.

Once a histone polypeptide chain has been synthesized, several of its lysine residues may be acetylated, and, as Candido and Dixon report (*ibid.*, 2015), although the sequence of the first

**Selecting Haploid Plant Cells**

BACTERIA lend themselves to sophisticated genetic analysis for several reasons, not the least of which are that they are haploid and they multiply quickly. Genetic analysis of higher plants and animals, by contrast, is hindered by the much longer life cycle and by the diploid nature of the somatic cells. If somatic cell geneticists could at will produce and maintain haploid cell lines, half their battle would be over, which explains why the report of Gupta and Carlson in next week's *Nature New Biology* (September 20) should be welcome reading for many.

Parafluorophenylalanine has for the past several years been used occasionally to induce the haploidization of one or two species of different genera of fungi, but Gupta and Carlson appear to be the first people to exploit this com-

pound to select and maintain cultures of haploid cells from higher plants. As a model system they established in culture cells of haploid and diploid strains of tobacco plants, and then added various concentrations of parafluorophenylalanine to the culture media. At concentrations which kill a callus of diploid cells a haploid cell callus continues to grow vigorously. It should therefore be possible to use this amino-acid to select haploid cells from populations of cells of varying ploidy, such as emerge in anther cultures. To date, of course, Gupta and Carlson have used only tobacco plant cells, but if the effect of parafluorophenylalanine is not specific to this species they may well have hit upon a generally useful method of obtaining haploid, higher plant, cell cultures.



twenty-five amino terminal amino-acids of trout testis histone III is identical to the sequence of the corresponding segment of calf thymus histone III, the pattern of acetylation of these two histones differs. In both calf and trout histone III, lysine residues 14 and 23 are acetylated but the lysine residues at positions 9 and 18 in some of the trout testis histone molecules are also acetylated.

The first twenty-two amino-acids in trout testis histone IIb<sub>2</sub>, which differs at several positions from the amino-acids of the corresponding segment of calf thymus histone IIb<sub>2</sub>, include four acetylated lysine residues, those at positions 5, 10, 13 and 18. Comparison of the amino-acids adjacent to acetylated lysine residues in trout testis histones III and IIb<sub>2</sub> reveals an interesting pattern. Acetylated lysine residues are either bordered on either side by amino-acids with short neutral side chains (glycine, threonine and alanine, the codons of which are related by single base changes) or the acetylated lysine residue is part of a Lys-Arg, Arg-Lys or Lys-Lys pair. Such sequences may well, therefore, provide part of the recognition signals which presumably dictate the sites at which specific acetylase can act. But, as Candido and Dixon point out, other factors such as the secondary structures of these proteins must be involved in the specificity of acetylation because, for example, lysine residue 47 in rabbit and calf histone I is not acetylated, even though it is preceded by a threonine residue and followed by an alanine residue.

Allfrey and Mirsky, Dixon and others, have postulated that the acetylation, methylation and phosphorylation of histones alter the ability of these proteins to react with DNA, and thereby play a part in the regulation of gene expression. The observation, made by Ryan and Cristofalo (*Biochem. Biophys. Res. Commun.*, **48**, 735; 1972), that as populations of cultivated human fibroblasts (WI-38 cells) age, the rate at which the cells are able to acetylate their histones decreases markedly, even though the ratio of histone/DNA per cell remains constant, can, of course, be accommodated by this hypothesis. But whether changes in the extent or pattern of acetylation of histones have any role in ageing remains to be seen.

## MEMBRANES

### Solid for Liquidity

from our Molecular Biology Correspondent  
CONSIDERING the weight of effort that has been brought to bear on the analysis of line shapes in n.m.r. and e.s.r. spectra of phospholipid bilayers, and the minute detail concerning the static and dynamic characteristics of the chains in these

bilayers that has been extracted, one might suppose that the point of diminishing returns has long been passed. All the same, another squeeze of the lemon has produced something new. Work from two laboratories has demonstrated that, depending on conditions, there is rapid translational diffusion of phospholipid molecules in the plane of the bilayer. Since the now celebrated demonstration by Frye and

Eddidin of protein antigens diffusing rapidly across a cell membrane, this result should perhaps occasion little surprise. Its demonstration, together with an evaluation of diffusion coefficients, is nevertheless of no little interest, and reinforces again the view, asserted in recent years, that real and model membrane systems in general behave as layers of fluid, within which proteins may float more or less freely.

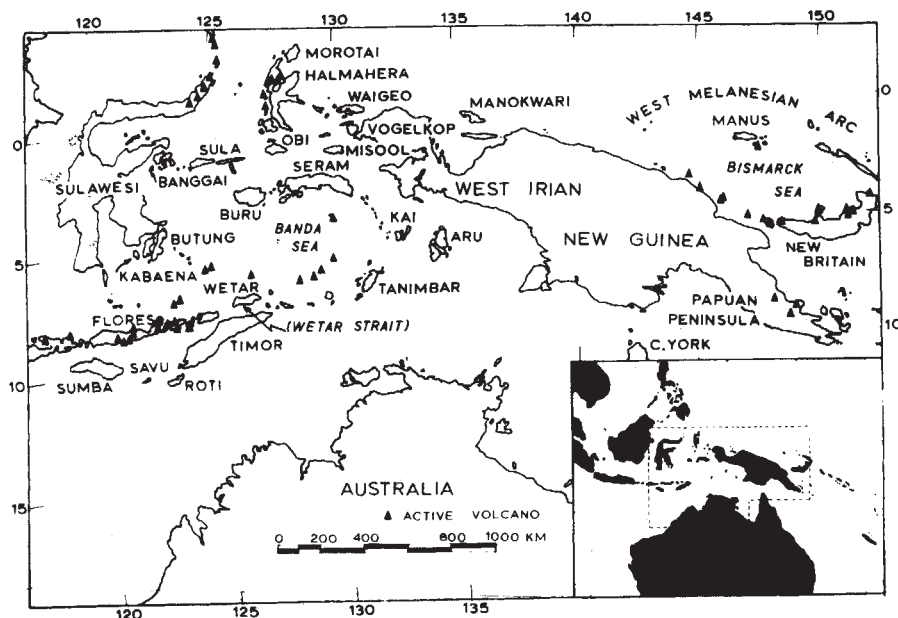
## Plate Tectonics in South-east Asia

As Tarling remarked in *Nature* (239, 38; 1972) two weeks ago, South-east Asia is a complex area which is little known from the point of view of plate tectonics. But with the publication of next Monday's *Nature Physical Science* (September 18) the situation will have been transformed by a magnificent interpretation of the tectonic development of eastern Indonesia in relation to the breakup of Gondwanaland, carried out by Audley-Charles, Carter and Milson of Imperial College, London. For what Audley-Charles and his colleagues have done is to marshal both geological and geophysical evidence for the evolution of Indonesia from the early Cretaceous to the present. Of course, only time will tell whether the interpretation is correct in detail; but what is certain is that the study by Audley-Charles *et al.* will for some time to come be regarded as the basic model against which detail will be judged.

The problem with South-east Asia stems from the complex geological history of the Banda Arcs (see map)—the Inner Banda Arc, which extends from the volcanic islands east of Flores through Wetar to the volcanoes in the Banda Sea south of Seram, and the Outer Banda Arc, which extends from Timor through Tanimbar, Kai and Seram to Buru. In general terms,

Audley-Charles *et al.* conclude that the Outer Banda Arc formed part of the Australian continental margin from at least the Permian, but that Seram, Buru and eastern Sulawesi became detached after the breakup of Gondwanaland—a conclusion which implies that these areas have undergone very large rotations, and that during the mid-Mesozoic central New Guinea must have lain to the east of Australia.

The subsequent history of the area, the geological manifestation of which is a series of orogenic events recorded in the Outer Banda Arc and eastern Sulawesi, is then seen in terms of a series of collisions between the northward-drifting Australian continent and a series of northward-dipping subduction zones. The first such collision took place between the late Cretaceous and the Eocene, after which renewed subduction began further north to allow the continuing northward motion of Australia. The second collision (with the new subduction zone) then occurred during the middle Miocene, by which time central New Guinea had separated from the east coast of Australia and had rotated into an east-west position. There then developed another subduction zone still further north, with which the Australian continent collided (the third collision) during the late Pliocene.



Devaux and McConnell (*J. Amer. Chem. Soc.*, **94**, 4475; 1972) have introduced a spin-labelled phosphatidylcholine derivative into planar bilayers, arranged in stacks, of native phosphatidylcholine. This kind of system, containing the spin-labelled derivative at low concentration, was used in earlier work from McConnell's laboratory to study the nature of hydrocarbon chain motion. In the new experiments, however, the spin-labelled phospholipid was introduced at rather high concentration, and incompletely mixed into the matrix, so that the molecules were distributed in large clusters, rather than randomly, in the bilayer. Unpaired electron spins close together interact, with gross broadening of the e.s.r. line widths, and this is what is observed in the clustered system. With time, however, the lines begin to sharpen, and after two days or so, the e.s.r. spectrum is not very different from that of a random system. Knowing, from microscopy, the diameter of the patches of the coloured, spin-labelled phospholipid, and the dependence of the line width on concentration, the evolution of the spectrum with diffusional dilution can be simulated, and the rate of spreading then gives the diffusion coefficient. This works out at about  $2 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ —a sizable value, although a good deal smaller than for molecules of this size in free solution. If the diffusion is treated as a hopping process between lattice points, this diffusion coefficient corresponds to about  $10^7$  jumps  $\text{s}^{-1}$ . This is not very different from McConnell's earlier estimates of the rotational isomerization rate of the hydrocarbon chains in the bilayer, or of rates of rotation of guest molecules.

With a similar objective in mind, and also using the shape of the e.s.r. signals from a spin label incorporated into an artificial bilayer, Sackmann and Träuble have arrived at a closely similar conclusion. Theirs, however, is an equilibrium method, and is related to that of Devaux and McConnell only in the technique of observation. They are also concerned with the differences in the structure of the bilayer above and below the transition temperature, at  $41^\circ \text{C}$  in their system, which can be observed as a discontinuity in a variety of physical parameters. By way of preamble (*ibid.*, 4482), they have used the e.s.r. spectrum, and the fluorescence and absorbance of dye molecules soluble in phospholipid, to follow the transition in a system consisting of spin-labelled androstane as a guest molecule in a dipalmitoyllecithin bilayer. The optical labels respond to the transition, largely in virtue of increased solubilization in the membrane above the transition temperature. This loosening of the lattice is also reflected in the diminution of the e.s.r. line widths from the spin label, in

consequence of increased freedom of tumbling. When the spin label is present in high concentration, a large line broadening effect occurs if the temperature is below the critical value. The results are analysed in a second paper (*ibid.*, 4492). Spin-spin interaction can elicit line broadening by two separate mechanisms, exchange (which requires van der Waals contact) and dipole-dipole interaction. The effects of these two perturbations on the line shapes are readily calculated, and comparison of simulated with observed spectra leads to the interpretation that exchange predominates, and decreases dramatically when the temperature is raised above the phase transition. In their third paper, Träuble and Sackmann (*ibid.*, 4499) show that the exchange efficiency obeys expectations in regard to its dependence on concentration of the unpaired spins and the temperature (to which it responds in a characteristic manner, different from that of dipole-dipole interactions). The exchange frequency is proportional to the number of collisions of spin-labelled molecules in unit time, and this in turn gives a diffusion coefficient of about  $10^{-8} \text{ cm}^2 \text{ s}^{-1}$ . This applies only to the temperature range above the transition, for below this the dependence of exchange on concentration is consistent with the onset of phase separation, the androstane forming substantial clusters within the phospholipid matrix.

Scandella, Devaux and McConnell (*Proc. US Nat. Acad. Sci.*, **69**, 2056; 1972) have used the same approach to

the observation of the diffusion of spin-labelled phosphatidylcholine in sarcoplasmic reticulum membrane, dispersed by sonication, but native in the sense that the calcium pump is still functional. The labelled lipid spontaneously diffuses into the bilayers, and only at very high concentration does it inhibit the ATPase. Above about  $40^\circ \text{C}$ , concentration broadening of the signals is dominated by exchange, as the temperature dependence shows. As before, the collision rate, and hence the transitional diffusion coefficient were determined, and the value of the latter, some  $10^{-7} \text{ cm}^2 \text{ s}^{-1}$ , is quite similar to that in vesicles of lipids derived from the same membrane. At low temperatures there is again evidence of phase separation. The rapid translational diffusion of phospholipid (which may move at 5 to  $10 \mu\text{m s}^{-1}$ ) is therefore a reality in a biological membrane under more or less physiological conditions. Scandella *et al.* point out, however, that different membranes will vary in this regard, and in some, in which the hydrocarbon chains appear to be much more extended and less flexible, translational diffusion will not be expected to operate on the same time scale.

#### STRONTIUM METABOLISM

### Fallout and Spin-off

from a Correspondent

THE human foetus discriminates between radioactive and stable strontium. This remarkable conclusion was reported in

## Recognition of Gravitational Radiation

A COMMUNICATION in next week's *Nature Physical Science* (September 18) points out a feature of gravitational radiation which could assist in the vital task of positive identification. So far the art of identification has consisted of what the author, W. L. Burke, calls "Holmesian sleuthing", in which one eliminates as impossible all more probable sources of disturbance to the detecting apparatus and concludes that gravitational radiation, however improbable, is the only remaining possibility and must be the truth. Weber's important experiments have observed disturbances in massive objects from which all other sources of disturbance have been eliminated. Because it is impossible to remove all other sources completely, it has been necessary to have more than one antenna and to observe coincidences between disturbances with a time lag between antennae as evidence for a travelling gravitational wave. Moreover, because only large sudden disturbances are observable, Weber's experiments have been able to reveal only wave fronts with a pulse-like structure.

Burke points out that gravitational waves have a unique feature permitting their identification. In the equation of motion of a weighted spring driven by a gravitational wave, the disturbance does not consist of a driving force, but appears as an extra term in the proper acceleration measured at the end of the spring—in fact as a pseudoforce. The second time derivative of the strain is not equal to the proper acceleration, but differs from it by this pseudoforce term. Only general relativity can so influence our ideas of inertia.

The effect of this feature is to alter the phase relation between the strain and the elastic force. Instead of being  $180^\circ$  out of phase for the input of a cosine wave, the proper acceleration has a phase shift dependent on frequency and with a peak at resonance.

Burke's communication shows that there is, in principle, an internal method for positive identification of gravitational waves with a single antenna. It is not restricted to pulse-like wave fronts, but identifies cosine waves and (by means of cross-correlation) arbitrarily structured disturbances.



a paper sent by Dr B. K. Borisov (Institute of Biophysics, Moscow) to the Second International Conference on Strontium Metabolism held at Glasgow and Strontian between August 16 and 19. Dr Borisov analysed the skeletons of seventy-seven fetuses (aged between 3 and 8 months) and forty stillborn infants. The normal full-term infant contains about 10% of the calcium ingested by the mother during pregnancy, 3% of the stable strontium and less than 1% of the radioactive strontium. Dr Borisov's paper suggested that dietary stable and radioactive strontium, being in different chemical forms, might not be equally assimilable.

Three important lessons emerged from the conference. First, the risk of leukaemia or bone cancer (the chief reason for interest in fallout) now seems to be smaller than has sometimes been supposed. Dr Marvin Goldman (University of California, Davis) reported on large scale experiments using beagles, which have useful anatomical, physiological and metabolic similarities to man. Studies involving the administration of  $^{90}\text{Sr}$ , at various dose levels, over a period of twelve years, have now produced reliable dose-effect curves in the low dose region which has been the scene of speculation and controversy for many years. Dr Goldman's findings show that the damage to be expected from fallout in the human body is much smaller than has been suggested in some well publicized estimates based on extrapolation from fewer adequate data.

Second, and not surprisingly, governments are losing enthusiasm for systematic monitoring of fallout. The British programme of human bone monitoring is being terminated. This decision was influenced by the work of a group led by Dr J. H. Marshall (Argonne National Laboratory), who described a mathematical model of strontium metabolism which seems capable, with modifications, of predicting bone levels of  $^{90}\text{Sr}$  from measurements on diet.

Third, the challenge of fallout has stimulated much important research in bone physiology and in metabolic disorders such as osteoporosis, as well as work on the mechanisms of intestinal absorption. The world's largest tracer experiment, though completely unplanned, still provides problems and opportunities for scientists and clinicians.

## ENTOMOLOGY

### Canberra Congress

from a Correspondent

SOME 1,500 entomologists from more than sixty countries met in Canberra between August 22 and 30 for the Fourteenth International Congress of

Entomology. The Australian National University provided excellent facilities, and Australian scientists, particularly those in the Entomology Division of the Commonwealth Scientific and Industrial Organization (CSIRO), whose head, Dr D. F. Waterhouse, was president of the congress, were responsible for the planning and smooth running of the programme.

Striking new discoveries are seldom reported to this type of gathering, but its programme did indicate interesting trends in contemporary entomology. Dr S. L. Tuxen, who retired from the office of President of the Permanent Committee after service of more than 20 years, pointed out that taxonomy, which had dominated most earlier meetings, now received little coverage (and comparatively small audiences for those papers that were included), even though all branches of entomology depended on the accurate determination of species. There were, however, many unofficial discussion groups among experts on different groups of insects, so this aspect of entomology was somewhat less neglected than the official programme might suggest.

The general impression was of a predominant interest in economic problems, with the largest audiences for symposia, often of a theoretical nature, on pest management and integrated control. At the opening of the plenary session, Dr E. F. Knipling (Agricultural Research Service, Beltsville, Maryland) discussed the significance of his own pioneer work, involving the use of sterile males to control pests in relation to biological control generally, and showed how repeated releases of large numbers of "beneficial" insects could be used almost as living insecticides. Dr P. W. Geier of CSIRO played a leading part in the organization of

several symposia and sections concerned with pest control, although the congress was brought down to earth by Professor M. J. May (Imperial College), who criticized the "life table—life system—pest management" approach, and suggested that it had so far made little practical contribution to the actual business of pest control and to agriculture generally. There was throughout the meeting a general realization of the limitations of the value of many insecticides, although, as might be expected in a body of people with practical experience of pests and insect-borne diseases, little of the "ban them all" attitude which is so prevalent in less-informed gatherings. At a symposium on "Insecticides and the Environment", Professor R. L. Metcalf (University of Illinois) and Professor H. T. Reynolds (University of California, Riverside) dealt constructively with the problems of developing and using new pesticides, and O. Starnes (Rockefeller Foundation, India) with the continuing need, even for DDT, in developing countries. Dr R. Goulding (Guy's Hospital Medical College) spoke of the significance (or lack of significance) of the universal traces of DDT and its metabolites in man, and Dr N. W. Moore (Monks Wood Experimental Station) reviewed the situation relating pesticides to wildlife conservation.

While the symposia dealing with general problems of pest management played to packed houses, the audiences for many of the afternoon sections, where those active in practical pest control described, often for the first time, their own contribution, were often disappointingly small. There was little apparent interest in laboratory studies in insect physiology, though insect behaviour, particularly as it relates to control, and to the use of pheromones,

## Lambda Phage DNA Replication initiated *in vitro*

IN *Nature New Biology* next week (September 20) Klein and Powling describe a cell-free system which supports the initiation of replication of lambda phage DNA, and, although it is early days yet, this system holds considerable promise for those intent upon elucidating the biochemistry of this event. Genetic investigations have revealed that two lambda genes, O and P, specify diffusible products that are required for the initiation of replication of the phage DNA. Mutants defective in either of these genes fail to replicate, but when *Escherichia coli* cells are doubly infected with one O gene mutant strain and one P gene mutant strain, complementation occurs *in vivo* and the phages replicate.

Lysates of such doubly infected cells, produced 7 min after infection, support

the initiation of lambda DNA synthesis as do, albeit to a lesser extent, mixtures of lysates of cells singly infected with one or other mutant phage. Moreover, when rifampicin is added to such lysates, DNA synthesis is significantly inhibited because new rounds of replication cannot apparently be initiated even though daughter DNA chains initiated before the addition of the antibiotic are elongated normally in its presence. RNAase, by contrast, does not significantly inhibit initiation of DNA synthesis.

Whether this means that the role of RNA polymerase in the initiation of lambda DNA replication is structural rather than enzymatic is one of the several questions which may be answered by further exploiting this cell-free system.

received a good deal of support, both at the symposia and at the closing plenary session where Professor J. S. Kennedy (Imperial College) spoke on the emergence of the study of behaviour as an independent discipline with an important contribution to make to applied ecology and pest control generally.

## MATHEMATICS

### Trends in Education

from a Correspondent

THREE years ago, at the first International Congress on Mathematical Education in Lyons, France, there were 600 participants. This past week (August 29 to September 2) at the second congress at Exeter University, there were 1,700 from seventy countries, and many were turned away for lack of accommodation. This is a measure of the extent to which interest in the subject has grown.

The advance that is most apparent is in the emphasis placed on those aspects of mathematical education concerned with communicating a working knowledge of how mathematics interacts with other subjects and on the external world: that is, a knowledge of how mathematics is applied. The congress programme was designed to view mathematical education within the context of the total education of the individual. Thus the plenary sessions were concerned not with specialized matters, but to place the subject within wider contexts, including the historical, sociological, and psychological, and also that of its relationship to the development of mathematics itself. A typical paper was by the anthropologist Dr Edmund Leach (University of Cambridge).

In his opening address, the chairman, Professor Sir James Lighthill (University of Cambridge), stressed that a specific characteristic of the British approach to mathematical education has been a close association between pure and applied mathematics, and a general predilection for teaching mathematics in a way that emphasized some of its applications. Sir James is much concerned with the contribution of mathematics to help in solving the world's problems. In some ways his attitude contrasts strongly with those mathematical educators concerned with more abstraction than ever before. No one denies the attractiveness of an appreciation of the beauty of mathematical structures and deductions, but the prevailing attitude at Exeter was for a deeper integration of mathematics into the total education of the individual.

Sir James suggested that educators might have most benefited their pupils when they had succeeded in giving a

feel for what was involved in the process of applying mathematics. This, he said, is the process of building a bridge between the abstract ideas and inferences of mathematics and the concrete problems arising in some field of application; and, he went on, it seems to be increasingly recognized that there may be more skill, more art, in that bridge building process than in the associated mathematical problem solving. This approach was taken up in two chief ways at the congress: in the thirty-eight working groups and in the national presentations by eighteen countries, at which there were classrooms of children being taught.

The working groups covered a vast range of topics, but, significantly, that most widely attended was concerned with the psychology of learning. Discussion here was much influenced by the work of the Swiss psychologist, Jean Piaget, whose former student, Joan Bliss, was group liaising secretary. For some who attended, the hope was that this might lead to the beginnings of a science of learning. The group on mathematics in developing countries considered such basic questions as the social relevance of the teaching of mathematics, and whether the traditional university institution was necessary in their countries.

During the past decade there has been a growth in the number of mathematical competitions, national and international. The best known is the annual

International Mathematical Olympiad organized at inter-governmental level, and held each year in one of the socialist countries, with some limited Western participation. The working group concerned with this discussed the use of competitions in the classroom, whether they could be fair, and what contribution they made at an international level. It was agreed to set up a working party to report on how maths competitions might be fostered, nationally and internationally; and an offer was made by the Awards Committee of the British Mathematical Association to act as an information clearing house.

## ELEMENTARY PARTICLES

### Still no Quarks

THE first results of yet another experiment to look for quarks, at the Intersecting Storage Rings at CERN, reveal no particle showing the characteristic features of a large mass and a charge of  $\frac{1}{3}$  or  $\frac{2}{3}$  of the electronic charge. So far,  $0.6 \times 10^9$  secondary particles produced in proton-proton collisions have been examined (Bott-Bodenhausen *et al.*, *Phys. Lett.*, **40B**, 693; 1972).

The CERN experiment shows that the cross-section for production of quarks with charge  $\frac{2}{3}e$  and mass up to 13 GeV is less than  $6 \times 10^{-34}$  cm<sup>2</sup>, and that the upper limit for  $\frac{1}{3}e$  particles up to 22 GeV is  $3 \times 10^{-34}$  cm<sup>2</sup>.

## In vitro Initiation of HeLa DNA Synthesis

ELUCIDATING the biochemistry of DNA replication is not a task for the faint-hearted; after almost two decades of research, precious little can be said about the way DNA is replicated even in organisms as simple as bacteria, and next to nothing is known about the process in eukaryotes. There are at last, however, encouraging signs of progress. As Kumar and Friedman, for example, report in *Nature New Biology* next week (September 20), they may well have detected a factor(s) which specifically promotes the initiation of DNA synthesis in preparations of isolated HeLa cell nuclei.

Experiments involving the fusion of HeLa cells that are at different stages of the cell cycle have led to the conclusion that the cytoplasm of HeLa cells, which are in the S phase and replicating their DNA, contains a factor which causes nuclei in the G1 phase of a cell cycle to enter into S and begin DNA replication. Kumar and Friedman have apparently reconstructed this system *in vitro*; they isolated nuclei from early and late G1 phase HeLa cells and then incubated them with the appropriate precursors and labels, together with

cytoplasm from one of several sources, namely early G1 cells, G2 cells, S phase cells or, as a control, buffered albumin. The only combination of nuclei and cytoplasm which led to a significant increase in the proportion of labelled nuclei making DNA was the late G1 phase nuclei-S phase cytoplasm combination. Cytoplasm from cells at other phases of the cycle did not promote DNA synthesis in late G1 phase nuclei, while nuclei from cells at stages other than late G1 did not respond to any of the cytoplasmic extracts.

The factor or factors in S phase cytoplasm that promote initiation of DNA synthesis in late G1 phase nuclei, assuming of course that the effect has nothing to do with changed DNA precursor pool sizes, is heat labile, resistant to pancreatic ribonuclease and withstands freezing and lyophilization. The hope is, of course, that S phase cytoplasm will prove to contain a specific initiator of DNA replication in receptive late G1 phase nuclei of HeLa cells, but it is as well to remember that as yet other less interesting interpretations of these data have not been rigorously excluded.



# The Seventh Pandemic of Cholera

B. CVJETANOVIC & D. BARUA

Communicable Diseases Division, WHO, Geneva

**The El Tor vibrio, responsible for the present cholera pandemic, has spread from Indonesia to the Far East, India, the Middle East, Africa and Europe, and may extend further.**

THE causative agent of the present seventh pandemic of cholera is the El Tor vibrio, so called after the El Tor quarantine camp on the Sinai Peninsula where it was first isolated in 1905 by Gotschlich from the gut of six pilgrims returning from Mecca. These pilgrims showed no ante or post-mortem evidence of cholera, but the vibrios were agglutinable with anti-cholera serum and resembled the true cholera vibrio in all the tests available at that time. Kraus and Pribram<sup>1</sup> later found the organism to be more akin to cholera-like vibrios (the so-called non-agglutinable or non-cholera vibrios) as it produced a soluble haemolysin. Many workers designated all haemolytic vibrios as El Tor vibrios until Shousha<sup>2</sup> and Gardner and Venkatraman<sup>3</sup> recommended the use of this designation exclusively for the haemolytic vibrios agglutinating with anti-cholera serum—in other words, those belonging to Gardner and Venkatraman's O-subgroup 1.

Not much importance was attached to this organism even after the discovery that it was responsible for a cholera-like disease of high infectivity but low morbidity and high fatality<sup>4</sup> in Sulawesi (Celebes) in Indonesia. Isolated instances of choleraic disease by El Tor vibrio were also reported from some countries in Europe and in the Near East<sup>5</sup>. This vibrio caused several localized outbreaks in the Indonesian archipelago in the 1940s and 1950s, but the circumstances that led to its leaving its domicile in 1960–61 to cause the seventh pandemic will probably never be known. Nor will it be known whether any of the previous six pandemics were caused by the El Tor vibrio as none of them were adequately investigated and the necessary knowledge was lacking until the time of the sixth pandemic.

## Distinguishing Characteristics

Although Pollitzer<sup>5</sup> considered it justifiable to designate *V. cholerae* and *V. eltor* as two distinct species, Hugh<sup>6</sup> proposed that both organisms be recognized as a single species of *V. cholerae* as they are similar in thirty positive and twenty negative characteristics and the differentiating features are rather infrasubspecific. These differentiating features may, however, be of epidemiological significance, and thus the El Tor vibrios are generally designated at present as *V. cholerae* biotype eltor.

The haemolytic property of El Tor vibrio which originally distinguished it from the classical cholera vibrio has now become of doubtful significance because most of the currently isolated strains of El Tor are not haemolytic by the classical or modified Greig test, although many of them show haemolytic activity when grown in heart infusion broth with 1% glycerol<sup>7</sup> or blood agar containing thioglycollate

and cystine<sup>8</sup>. Of several other differentiating features, direct haemagglutination, resistance to 50 units of polymyxin B in disks and to cholera phage group IV at routine test dilution are useful for characterizing El Tor vibrios.

The El Tor vibrio survives longer in the environment than the classical cholera vibrio. In India, biotype eltor has been isolated from various natural water sources, usually in the absence of the disease in the neighbouring community, while strains of classical cholera vibrio have never been isolated from such water sources in the absence of cases<sup>9,10</sup>. In laboratory studies too, El Tor vibrios have been found to survive longer in water and food than classical vibrios<sup>11,12</sup>. This ability of the El Tor vibrio to survive longer outside the human host has enabled it to be detected by bacteriological examination of nightsoil and latrine samples<sup>13,14</sup>. In an area with simultaneous El Tor Ogawa and classical Inaba infection, it was possible to isolate El Tor Ogawa ten times more frequently from nightsoil than classical Inaba<sup>15</sup>.

The minimum inhibitory concentration of vibriostatic or vibriocidal antibiotics has also been found to be relatively greater for El Tor vibrios<sup>16</sup>.

## Infection with El Tor

The El Tor vibrio has been found to cause many more mild and asymptomatic infections than typical cases: the case-to-infection ratio has varied from 1:5 or 1:10 in one area, 1:36 in another and 1:99 in yet another, depending on the density of the population, the habits of the people, the degree of contamination of the environment and, of course, on the quality and thoroughness of surveillance. With the classical biotype, the case-to-carrier ratio is about 1:4 in Bangladesh<sup>15</sup>. The duration of the El Tor carrier state in asymptomatic contacts is generally about a week. A few examples of cases that have become long-term carriers after recovery have been described, and all are due to the El Tor vibrio. One such person (Cholera Dolores) is still intermittently vibrio-positive on stool examination and on duodenal intubation 9 years after her illness, and the organism is probably harboured in her gall bladder.

Although mild and asymptomatic infection is more common with El Tor, severe illness, clinically indistinguishable from that caused by the classical biotype, does occur. The clinical outcome of the infection, however, depends on many factors, one of which is the infective dose. While the attack rate by El Tor vibrio has varied in Asia from 2 to 20 per 10,000, in certain localized areas of Africa, an attack rate of 800 to 1,000 per 10,000 has been seen.

## Spread of Infection

The ability of this sturdier organism to survive longer in the environment and to cause a large number of mild and asymptomatic infections has certainly been a contributing factor in the emergence of the present pandemic. However, in view of the fact that the presence of the El Tor vibrio and outbreaks of cholera due to this organism were noted much earlier in Indonesia<sup>4</sup> and elsewhere in South-East Asia, it is difficult to explain why there was no pandemic spread of the disease before 1961. It is likely that international and

even inter-island travel and communications were not sufficiently developed in the first part of the twentieth century to permit the extension of El Tor cholera from its endemic foci. It is in the second half of this century that rapid motor boats have been introduced in island-to-island traffic, and that there has been a boom in sea, air and road traffic, freeing cities and villages from their isolation. The rapid development of communications and means of transport inside countries and on an international scale was not accompanied by a rise in levels of personal and environmental sanitation. In many countries of South-East Asia, the population explosion, the rural exodus and civil unrest have caused such a disturbance that the standards of sanitation have actually deteriorated. In these areas some religious habits such as ritual immersions and cleansing by hand after defaecation have also contributed to the introduction and spread of infection in certain population groups. Only countries with low levels of environmental sanitation and education have been invaded and have suffered serious outbreaks.

The reporting of cholera is far from complete, but nevertheless the chronological extension of the seventh El Tor cholera pandemic is known (Fig. 1). After spreading from its endemic focus in Indonesia to the countries in the Far East, the El Tor vibrio invaded areas in the Indian sub-continent where the classical *V. cholerae* was endemic and spread further westward to the Middle East, Africa and Europe.

## Distribution of Serotypes

During this pandemic, the distribution of the serotypes Inaba and Ogawa has followed an interesting pattern. In the East, the pandemic spread began with Ogawa in almost all countries (except Laos) but shortly afterwards the serotype changed to Inaba in the Republic of Vietnam, Hong Kong, Cambodia and Thailand. In 1969, Inaba invaded Laos. In Malaysia and Indonesia, both the serotypes have been isolated, although one or the other predominates at different times. In other countries, particularly in the Philippines, Burma and India, the Ogawa serotype remains the predominant strain. In most parts of India, classical *V. cholerae* Inaba was replaced by biotype El Tor Ogawa. In Bangladesh the classical *V. cholerae* is still the predominant agent though the serotype has recently changed from Inaba to Ogawa. Some El Tor Ogawa strains have also been isolated, particularly in the eastern part of the country, but the El Tor vibrio has failed to replace the classical biotype here as it did in India.

Inaba strains have been isolated in Iran<sup>17</sup> and this serotype has also recently been found in the Astrakhan area of the USSR, Jordan, Israel, Turkey and the Arabian Peninsula, as well as on the other side of the Red Sea in the French

territory of the Affars and the Isaas, Somalia, Ethiopia and Kenya. Ogawa strains, on the other hand, were isolated from Iraq in 1966 and, since then, from Lebanon, Syria, Odessa and Kerch in the USSR, Czechoslovakia, different countries in North, West and Central Africa, and the Iberian Peninsula. This serotype has also been isolated in the northern part of Uganda and in the Lake Rudolf area of Kenya.

Recently, Inaba strains have appeared in some countries of West Africa, such as Cameroon, Liberia and Niger. This is probably an example of sero-conversion similar to that seen earlier in Cambodia, the Republic of Vietnam and Thailand. At the end of December 1971, Inaba strains invaded Angola.

## Future Trends

It took a few years for cholera to become established in Asia before invading the continents of Europe (USSR and Czechoslovakia) and Africa in 1970. The incursion into Eastern Europe was halted in 1971 but cholera spread into the Iberian Peninsula and invaded more countries of Africa, extending southwards beyond the Equator.

While cholera seems to have been controlled in Europe because of the higher standards of sanitation and medical services, its control in developing countries, especially in Africa, is difficult. Many lives, however, have been saved by modern treatment, which consists of replacing the lost fluid by intravenous and oral solutions, the need for which can be reduced by suitable antibiotics.

Although, in this jet age, infection can be introduced into any country, cholera will not be able to establish itself in countries with a reasonable level of sanitation, whereas in areas of poor personal and community hygiene an extension of the disease may be practically inevitable. The future evolution of the pandemic is difficult to predict, but it could well extend to developing countries in Central and South America.

Although extensive research activities have led to considerable progress in the understanding of the mechanism and immunity of the disease and in its diagnosis and treatment, no medical means has yet been found of combating cholera in the affected areas and preventing the further spread of the seventh pandemic. The improvement of standards of living and sanitation in the receptive areas remains the only effective measure.

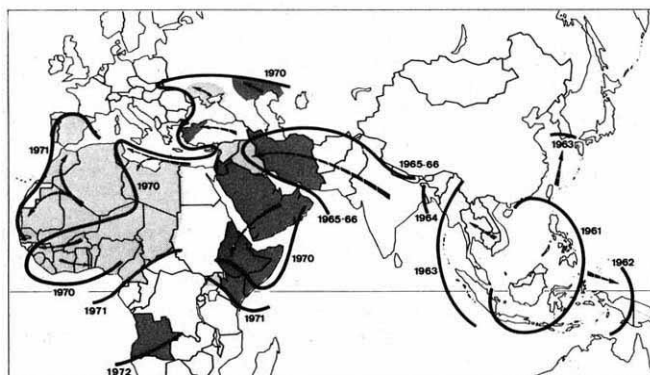


Fig. 1 Trends of global spread of cholera (1961–May 1972) and predominating serotype distribution during recent years. Light shading, Ogawa; dark shading, Inaba.

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# Laser Compression of Matter to Super-High Densities: Thermonuclear (CTR) Applications

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Hydrogen may be compressed to more than 10,000 times liquid density by an implosion system energized by a high energy laser. This scheme makes possible efficient thermonuclear burn of small pellets of heavy hydrogen isotopes, and makes feasible fusion power reactors using practical lasers.

THERMONUCLEAR burning occurs extraterrestrially in stars and terrestrially in nuclear explosions<sup>1</sup>. The specific thermonuclear burn rate is proportional to density

$$\dot{\phi} \sim \rho \bar{\sigma} v$$

where  $\dot{\phi}$  is the fractional burnup,  $\rho$  is the density, and  $\bar{\sigma} v$  is the Maxwell velocity-averaged reaction cross-section. Consequently, except at high fuel depletions, the thermonuclear energy production at a fixed ion temperature is proportional to the Lawson number, a product of density and confinement time<sup>2</sup>. In conventional controlled thermonuclear reactor (CTR) approaches, the density is limited by material properties, and the objective is to achieve sufficiently long confinement times by the use of electromagnetic fields<sup>3</sup>. In the laser-fusion approach to CTR, the objective is to achieve sufficiently high fuel densities, while the confinement time is determined by the inertia of matter. Spherical compression of  $10^4$ -fold via the laser implosion scheme described here reduces the laser energy required for CTR by more than one thousand-fold, from more than  $10^8$ – $10^9$  J—which is so large as to be currently impractical—to  $\sim 10^5$ – $10^6$  J, assuming laser and thermal/electric efficiencies of 10% and 40% respectively<sup>4</sup>. One kJ of laser energy may be sufficient to generate an equal thermonuclear energy.

## Compression

Hydrogen in the centre of the Sun is believed to exist at more than one thousand times liquid density, and at pressures greater than  $10^{11}$  atmospheres (temperature  $\sim 1$ – $2$  keV)<sup>5</sup>. These pressures are maintained gravitationally by the overlying enormous solar mass,  $\sim 10^{33}$  g. Matter in the cores of white dwarf stars is believed to exist at more than  $10^5$  g cm<sup>-3</sup>, and at pressures greater than  $10^{15}$  atmospheres<sup>6</sup>.

The electrons in white dwarf cores are Fermi-degenerate, so the pressure is a minimum determined by the quantum mechanical uncertainty and exclusion principles<sup>7</sup>. The pressure of dense hydrogen with Fermi-degenerate electrons is<sup>8</sup>

$$P = \frac{2}{3} n_e \epsilon_F \left[ \frac{3}{5} + \frac{\pi^2}{4} \left( \frac{kT}{\epsilon_F} \right)^2 - \frac{3\pi^4}{80} \left( \frac{kT}{\epsilon_F} \right)^4 + \dots \right]$$

where  $n_e$  is the electron density;  $\epsilon_F = \frac{h^2}{8m} \left( \frac{3}{\pi} n_e \right)^{2/3}$  is the Fermi energy;  $kT$  is the thermal energy;  $h$  is Planck's constant, and  $m$  is the electron mass. At  $10^4$  times liquid density ( $n_e = 5 \times 10^{26}$ ), the minimum hydrogen pressure occurs when  $kT \ll \epsilon_F$ , and is  $\sim 10^{12}$  atmospheres.

## Pressure: Implosion, Ablation

To compress hydrogen on Earth to these stellar densities, the required pressures must be generated by means other than gravitational. The pressures generated mechanically or chemically are generally limited to  $\lesssim 10^6$  atmospheres by the strengths of chemical bonds, although chemical explosive pressures have been multiplied from less than  $10^6$  to more than  $10^7$  atmospheres by implosion. The pressure applied to an implosion system does  $PdV$  work generating kinetic energy which is converted near isentropically to internal energy concentrated in the compressed volume. Because pressure is energy per unit volume, the maximum average pressure equals the applied pressure multiplied by the compression ratio. Additional pressure multiplication occurs near the centre because of convergence effects<sup>9</sup>. The pressure multiplication factor may be increased by implosion of hollow spheres, since the externally applied pressure acts over a larger volume<sup>10</sup>.

Laser light has been focused to intensities greater than  $10^{17}$  watts cm<sup>-2</sup> (ref. 11). At such intensities the "light pressure" (momentum flux) is almost  $10^8$  atmospheres ( $P \approx I/c$  where  $I$  is the intensity, and  $c$  the velocity of light). Much higher pressures can be generated with intense light by a combination of ablation and implosion. The momentum flux (and pressure) associated with laser-driven ablation is much greater than that of the light for the same reason that matter-ejecting rockets develop greater thrust than photon-drive rockets of equal power. Typical matter velocities associated with laser intensities of  $10^{17}$  W cm<sup>-2</sup> are  $10^8$  cm s<sup>-1</sup> ( $\sim$  sound speed at temperature of 10 keV), or  $\sim 3 \times 10^{-3} c$ . Hence the momentum flux and pressure, which are proportional to laser energy flux

divided by reaction velocity, can be increased by several hundred-fold to more than  $10^{10}$  atmospheres. The ablation pressure can then be further multiplied to more than  $10^{12}$  atmospheres by a laser-driven ablative implosion in which high compressions occur.

## Pulse Shape

The Fermi-degenerate state—which minimizes the required implosion pressure—may be achieved by shaping the laser pulse in time. When implosion begins, laser power is set so that the initial shock speed in the imploding matter is comparable to sound speed (pressures of  $10^5$ – $10^6$  atmospheres) and subsequently so that the compression is near-isentropic; the hydrodynamic characteristics intersect only near the centre of the sphere. Owing to the extreme convergence effects, and by adjusting the pulse shape so that the characteristics intersect just before the centre is reached, a small fraction of the pellet mass in the central region is compressed and strongly heated, producing thermonuclear ignition. The laser power history which generates an optimal, isentropic compression of a degenerate hydrogen sphere is approximately\*

$$\dot{E} = \dot{E}_0 \tau^{-s}$$

where  $\tau = 1 - t/t'$ ,  $t$  is time,  $t'$  (which is  $> t$ ) is the transit time to the centre of the sphere of the initial shock (generated by appli-

cation of  $\dot{E}_0$ ),  $s = \frac{3\gamma}{\gamma+1} = 15/8$  for dense hydrogen with degenerate

electrons ( $\gamma = 5/3$ ). Such a pulse shape may be generated with sufficient accuracy with a practical laser system. Implosion calculations show that the optimal power history can be satisfactorily approximated by a sequence of about ten pulses. Starting with the final shortest, most intense pulse (which need not be accurately shaped) the preceding pulses can be generated with sufficient accuracy with beam splitters, attenuators, and optical paths of various lengths. More sophisticated pulse shaping schemes are probably feasible.

## Symmetry

To implode matter to high densities the implosion pressure must be applied with sufficient symmetry, both spatially and temporally, and hydrodynamic instabilities must be adequately controlled. In compression of a sphere by  $10^4$  times, the radius decreases rather more than 20 times. If, after compression, spherical symmetry is required to within  $1/2$  the compressed radius—or  $1/40$  the initial radius—then the implosion velocity (and time) must be spatially uniform (and synchronized) to better than one part in 40, or a few per cent. In general, for a spherical implosion in which the ratio of initial to final volumes is  $\eta$ , and in which the tolerable error in the final radius  $r$  is  $wr$ , the associated tolerable fractional error in the product of velocity and time is

$$\frac{\Delta(vt)}{vt} \simeq \frac{w}{\eta^{1/3}}, \eta \gg 1$$

Implosion errors may be reduced to 10–20% by a many-sided irradiation system, consisting of beam splitters, mirrors, lenses,

\* Computer calculations of spherical implosions show that this power history generates a near optimal pressure history, and that this pressure history is (for a Lagrangian surface):

$$P = P_0 \left( \frac{h}{h_0} \right)^{2/3} \frac{S}{S_0}, \quad h = \int_0^R \rho dr$$

It may be shown analytically (via the hydrodynamic characteristics) that this pressure history also generates an optimal compression of a plane slab (where  $h=h_0=\text{const.}$ ). As expected  $P \sim \dot{E}^{2/3}$ , since  $\dot{E} \sim P \times \text{velocity}$ , velocity  $\sim P^{1/2}$ .

and other optical elements. For example, the entire surface of a sphere may be irradiated—with an intensity variation less than  $\pm 20\%$ —by 6 beams oriented along the 6 Cartesian directions, each focused with  $f/1$  optics to a point about one radius beyond the centre of the sphere, and with overlapping edges blocked out. The intensity variation may be reduced by use of more beams. The error of 10–20% can then be reduced to less than 1% by means of an atmosphere (generated by ablation with a laser prepulse) extending to several pellet radii with density slightly greater than the critical density (at which the laser frequency equals the plasma frequency). Laser light is absorbed and heats electrons in the outer atmosphere by inverse bremsstrahlung and plasma instabilities<sup>12</sup>. Asymmetries are reduced during energy transport by electrons through several mean free paths of atmosphere to heat the surface of the pellet. In addition, each point on the relatively small pellet is heated by, and averages over, a large fraction of the hot absorbing region in the outer atmosphere.

## Stability

The implosion of the pellet by diffusion driven ablation-generated pressures is hydrodynamically stable, except for relatively long wavelength surface perturbations which grow too slowly to be damaging. The amplitude of a perturbation on the droplet surface grows during implosion as  $A_0 e^{-\sigma t}$ , where  $A_0$  is the initially present amplitude of surface roughness, and

$$\sigma^2 = -ak + \frac{k^2 P_A}{\rho}$$

for acceleration  $a$ , wavelength  $\lambda = 2\pi/k$ , ablation pressure  $P_A$ , and density  $\rho$ . The first term  $ak$  is associated with the well-known Rayleigh-Taylor instability<sup>13</sup>. If  $\Delta x$  is the thickness of a shell of matter, then using  $a = P_A/\rho \Delta x$  (from  $F = ma$ ),  $\sigma^2$  is positive (implying stable acceleration) for  $\lambda \lesssim 2\pi \Delta x$ . In part, ablative stabilization occurs because the peaks of surface perturbations are effectively closer to the heat source (radius at which the critical density occurs) so that the temperature gradient is steeper. Consequently more rapid ablation occurs, and higher pressures are generated which reduce the amplitude of the perturbation. “Fire polishing” may also be a significant mechanism.

In the compression scheme which has been described, the spherical pellet is accelerated inwardly by the reaction forces associated with the outwardly expanding ablated material. In effect, this scheme is a spherical ablation rocket implosion system, externally energized by an optimally power-programmed high energy laser.

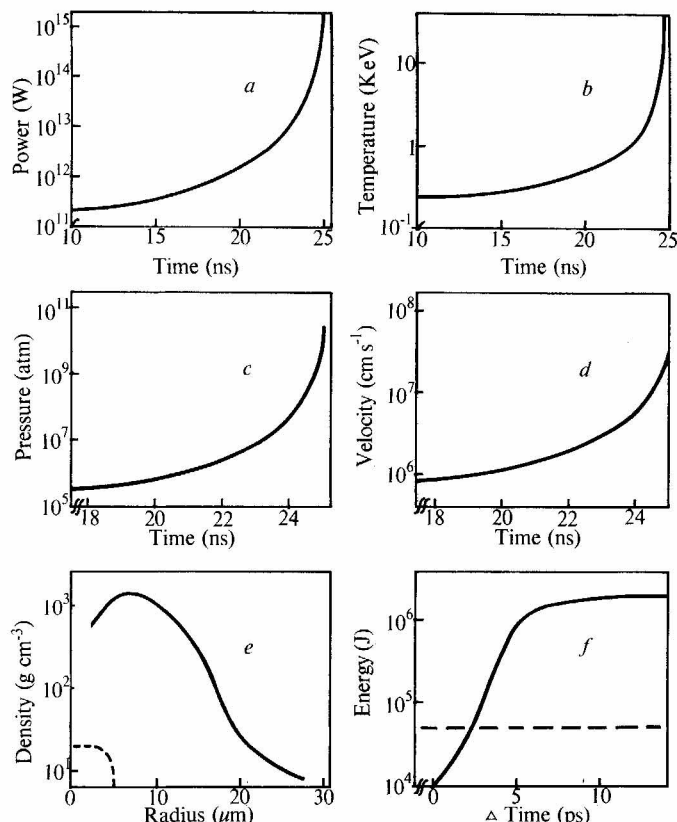
## Thermonuclear Burn

The inertial confinement time of a sphere of hot plasma is proportional to the radius divided by the sound speed. Therefore since the burn rate is proportional to density, the burn efficiency is proportional to the product of the density and radius,  $\rho r$ . In spherical compression  $\rho r$  increases because the density increases more rapidly than the radius decreases. At constant  $\rho r$ , the spherical mass is inversely proportional to the density squared.

Spherical compression reduces the minimum pellet mass and laser energy required for efficient thermonuclear burn. In addition, very high spherical compressions produce a product of radius and density so large that the energetic charged particles from the fusion reaction are absorbed within the dense pellet†, and the ion-electron coupling time becomes

† In DT at 10 keV electron temperature, the effective range of the 3.6 MeV alpha particles produced by DT fusion is  $\sim 0.3 \text{ g cm}^{-2}$ . A liquid density DT sphere with this  $\rho r$  has a mass of 3 g, and requires  $3 \times 10^9 \text{ J}$  to heat to 10 keV. At  $10^4 \times$  liquid density and the same  $\rho r$ , the mass and energy are reduced by  $10^8$ -fold.





**Fig. 1** Computer calculations of microexplosion resulting from 10,000-fold compression of a fusion pellet. A 60 kJ, 1 μm, laser is assumed to implode a 0.4 mm radius sphere of equimolar deuterium/tritium. *a*, Laser power. *b*, Electron temperature. *c*, Pressure. *d*, Velocity of shell. *e*, Density (—) and ion temperature (---) during thermonuclear ignition. *f*, Energy production; —, fusion; ---, laser.

shorter than the inertial confinement time<sup>14</sup>. The resulting self-heating further reduces the laser energy required for effective fuel ignition. Compression also provides a means of heating the fuel ions to ignition temperatures. Except in very low density matter, only electrons are significantly heated by absorption of laser light<sup>15</sup>. These considerations preclude CTR applications with uncompressed pellets using practical lasers. The advantages of compression are partially offset by energy losses associated with escaping ablated material. These losses are related to the implosion and blow off velocities by the rocket equation.

## Computer Calculations

Laser compression and fusion have been explored with the LASNIX computer program. LASNIX simulates by a Lagrangian finite difference scheme a wide variety of physical processes including hydrodynamics (by the von Neumann-Richtmeyer technique); (flux-limited) spatial energy transport and cross-field coupling between thermal ions and photons and up to 50 velocity groups of electrons; thermal radiation generation and absorption via bremsstrahlung and bound-free processes; laser-matter interaction via inverse bremsstrahlung and a variety of non-linear processes, involving appropriate electron spectral inputs to the electron velocity groups for each interaction (determined for non-linear processes by plasma simulation code studies); thermonuclear burn, including non-local time-delayed transport and appropriate electron-ion couplings of charged fusion reaction products. Realistic properties of matter are utilized, including equations of state, such as opacities, pressures and specific heats and transport coefficients which take into account nuclear coulomb, degeneracy and partial ionization effects.

Computer calculations of a 10,000-fold compression of a fusion pellet and the resulting thermonuclear microexplosion are shown in Fig. 1. In these calculations a 60 kJ, one micron wavelength laser implodes a 0.4 mm radius spherical liquid drop of equimolar deuterium-tritium (DT) located in a vacuum chamber. A laser prepulse ablates the pellet generating an atmosphere of DT which extends to a radius of ~1,000 microns with a density greater than  $4 \times 10^{-3}$  g cm<sup>-3</sup>. The applied laser power then optimally increases from ~10<sup>11</sup> to ~10<sup>15</sup> W in ~20 ns (Fig. 1a).

The laser light is absorbed in the outer atmosphere, heating electrons. In this calculation the electrons are assumed to be strongly coupled by possible plasma instabilities to form a near Maxwellian distribution. These hot electrons heat the atmosphere to electron temperatures which increase from ~10<sup>7</sup> to ~10<sup>8</sup> K during the implosion (Fig. 1b). The surface of the pellet is heated and ablated, generating pressures which increase optimally from 10<sup>6</sup> to 10<sup>11</sup> atmospheres (Fig. 1c). The many order of magnitude increase in implosion pressure occurs during the transit time of the initial shock to the droplet centre, so that the unablated outer part of the pellet is gradually compressed into a spherical shell with density > 100 g cm<sup>-3</sup>, while at the same time this shell is inwardly accelerated to velocities which increase from 10<sup>6</sup> to  $3 \times 10^7$  cm/s (Fig. 1d). As the internal pressures become larger than the ablation pressures, the rapidly converging shell slows down and is compressed, still near-isentropically at sub-degeneracy temperatures, to densities greater than 1,000 g cm<sup>-3</sup>. At the same time the low density non-Fermi degenerate central region is compressed by the shell to densities approaching 1,000 g cm<sup>-3</sup>, and heated in the process to ion and electron temperatures greater than 10<sup>8</sup> K, initiating thermonuclear burn (Fig. 1e). About 1,800 kJ of fusion energy is produced in less than 10<sup>-11</sup> s (Fig. 1f). Since a 60 kJ input of laser light was used, net electrical energy production would be possible with a 10% efficient laser and a 40% thermal-electric efficiency.

Calculations with non-Maxwellian electrons and linear electron coupling show that suprathermal electrons generated by laser plasma instabilities preheat the fuel during compression and effectively decouple from the atmosphere. Decoupling of thermal electrons may also occur<sup>16</sup>. These effects are worst with long wavelength lasers, such as CO<sub>2</sub>, because absorption occurs at a lower density. Generation of suprathermal electrons can be minimized by using inverse bremsstrahlung absorption of the laser light. The instability thresholds can be increased, and inverse bremsstrahlung absorption enhanced, by use of ultraviolet lasers and by seeding the pellet with small amounts of high Z material (~0.1 atom %). Decoupling limits the maximum ablation pressure. This can be compensated for by use of hollow pellets if suprathermal electron preheat is avoided. In non-Maxwellian linearly coupled calculations with ultraviolet lasers and seeded pellets, results similar to those described above have been achieved.

Fig. 2 shows the scaling of the gain,  $G_L$  (ratio of fusion energy to laser light energy) with compression and laser light energy. Gains approaching 100 are predicted for laser energies of 10<sup>6</sup> J. At compressions less than ~10<sup>3</sup>, the gain increases strongly with increasing compression because of increasing burn efficiency and alpha particle self-heating of the fuel. The gain decreases with compressions much greater than 10<sup>4</sup> because of depletion (of the DT) and because ablative energy losses increase and the energy of compression (against degeneracy pressures) becomes dominant.

The electrical gain,  $G_e$  (ratio of electrical energy outputted to electrical laser pumping energy), is

$$G_e = G_L \phi_t \phi_L$$

where  $\phi_t$  is the power plant thermal efficiency and  $\phi_L$  is the laser pumping efficiency. If  $\phi_t = 40\%$  and  $\phi_L = 10\%$ ,  $G_e > 1$  if  $G_L > 25$ , which occurs for a laser energy,  $L$ , less than 100 kJ for  $\eta = 10^4$

(Fig. 2). Also  $< 1$  kJ of light energy is sufficient to generate an equal quantity of thermonuclear energy, if optimally employed (Fig. 2).

## Pellet Cost

Cheap fuel pellets are required for commercial power production. Ten million joules of electrical energy is currently worth roughly one cent, and most of this cent must be used to pay capital and operating costs of the power plant. In the scheme described here the pellet may be a droplet of DT (or deuterium, using multi-megajoule lasers) ejected from a sophisticated eye dropper and spheroidized by surface tension and viscosity effects, while freely falling in an evacuated drop tower. The pellet may also be a hollow sphere of fusion fuel (although very small ratios of shell thickness to radius are precluded by symmetry and stability requirements). Hollow pellets require lower peak laser powers (but not less laser energy) and may be advantageous for lasers with wavelength  $\geq 1 \mu\text{m}$  (because of electron decoupling effects). The high cost of tritium can be overcome by regenerating the consumed tritium with neutron reactions in lithium blankets, or by burning essentially pure deuterium (with only a small tritium content).

## Reactor Economics

Thermonuclear microexplosions producing of the order of  $10^7$ – $10^8$  J (5–50 pounds TNT equivalent) are suitable for commercial power production. Multigigawatt-electric average power levels may be achieved by burning about 100 pellets per s, perhaps 10 per s in each of 10 explosion chambers.

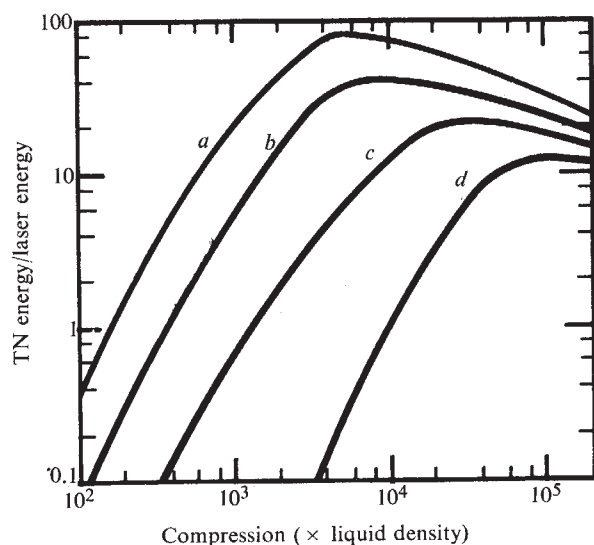


Fig. 2 Energy gain plotted against compression: a,  $10^6$  J; b,  $10^5$  J; c,  $10^4$  J; d,  $10^3$  J.

The electrical power  $P$  is related (through energy conservation) to the laser energy,  $L$ , explosion frequency,  $N$ , gain  $G_L$ , thermal-electric efficiency  $\phi_t$ , and laser efficiency  $\phi_L$  by

$$P = LN(G_L\phi_t - \phi_L^{-1})$$

where we assume the laser is electrically pumped and the hot gas exhausted from the laser is not used to generate electricity. It is desirable that  $G_L\phi_t \geq 2\phi_L^{-1}$  to avoid marginal designs. Hence, if  $\phi_t = 0.4$ ,  $\phi_L = 0.1$ , then  $G_L$  must be greater than 50, which occurs for  $L \sim 10^5$ – $10^6$  J (Fig. 2). Then if  $P = 10^9$  W,  $L = 10^6$  J and  $N = 100 \text{ s}^{-1}$ .

The explosive impulse from a small fusion explosion is much smaller than that from a TNT explosion of the same energy, because the fusion explosive weight is  $< 10^{-6}$  that of the TNT explosion, and the impulse generated is proportional to the square root of the explosion debris mass. A specially designed explosion chamber is required, however, to withstand the neutrons, X-rays, and hot plasma generated by the fusion explosion. Such an explosion chamber appears to be feasible<sup>17,18</sup>.

Conventional thermal cycles may be used to generate electricity if fusion neutrons are absorbed in lithium blankets, and the hot plasma cooled to manageable temperatures<sup>17,18</sup>. If relatively large DT pellets or essentially pure deuterium pellets are burned (requiring a multi-megajoule laser energy), the fusion neutrons will deposit much of their energy in the fuel plasma, which may be expanded against a magnetic field, to convert much of the fusion energy directly to electricity<sup>19,20</sup>, achieving very high electricity generation efficiencies. With deuterium pellet burning, lithium utilization and tritium storage and cycling may be greatly reduced. Net tritium is generated by deuterium burn.

Hybrid reactors, in which the 14 MeV DT neutrons which escape from the explosion chamber are used to fission natural uranium, or thorium, may generate more energy than used to pump the laser even with low efficiency, low energy lasers, such as 1% efficient, 10 kJ Nd glass lasers.

## Problem of Realization

Important areas requiring future study include suprathermal electron coupling and pellet preheating; effects of magnetic fields generated by laser driven gradients in the plasma temperature and density; engineering design of a sufficiently cheap reactor system which will function for more than  $10^{10}$  micro-explosions; and development of the required lasers.

We thank Edward Teller for critical discussions, and C. Haussmann, P. Moulthrop, A. Biehl and R. Hirsch for advice, support and encouragement. J. DeGroot and J. Katz have made important contributions to our understanding of laser-plasma interactions and plasma transport phenomena. J. Nuckolls and L. Wood wish to dedicate their portion of this work to the memory of their young colleague, Robert Dennis Wilson.

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# Specific IgG mRNA Molecules from Myeloma Cells in Heterogeneous Nuclear and Cytoplasmic RNA containing Poly-A

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**Ribonucleic acid containing A-rich sequences has been isolated from the nucleus and cytoplasm of mouse plasmacytoma cells (5563). Injection of this RNA into *Xenopus* eggs and oocytes has demonstrated that both the nuclear and cytoplasmic RNA contains messages for the immunoglobulin heavy and light chains.**

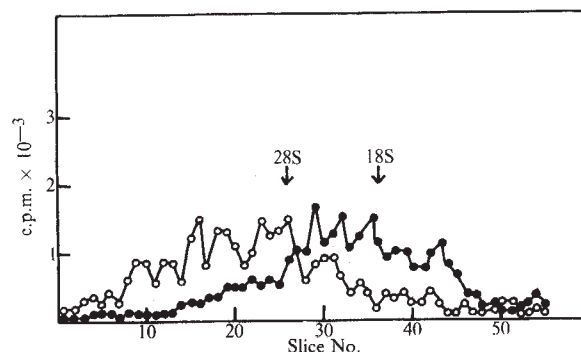
THE role of heterogeneous nuclear RNA (HnRNA) as a precursor to the cytoplasmic messenger RNA (mRNA) has been intensively researched<sup>1-3</sup>. The identification of an adenosine-rich sequence in RNA attached to both the HnRNA and the cytoplasmic mRNA of eukaryotes has further suggested the possible mRNA precursor role of HnRNA<sup>4-7</sup>. Although the RNA associated with the A-rich sequences has been characterized in several systems, it has not, except possibly for haemoglobin, been shown to contain messenger activity<sup>8</sup>.

Murine plasmacytoma cells (myeloma 5563 clone 34a, gift of E. Garbutt and J. Welstead) adapted to continuous *in vitro* culture produce large amounts of the four chain (H<sub>2</sub>L<sub>2</sub>) 7S immunoglobulin<sup>9</sup>. In this study, the RNA containing A-rich sequences has been isolated from both the nucleus and cytoplasm of myeloma cells and shown to be translated into heavy, light and possibly several combined forms of immunoglobulin chains when injected into the eggs or oocytes of *Xenopus laevis*. These results indicate that HnRNA is a precursor of mRNA and that A-rich sequences are indeed associated with mRNA and potential mRNA. Nuclei and cytoplasm were separated by treating cells treated with 0.7% 'NP-40' detergent at 4° C<sup>10</sup>. Under these conditions there is little loss of <sup>3</sup>H-thymidine from the nuclei, and electrophoretic analysis of the nuclear RNA fails to reveal 18S ribosomal RNA. This result indicates that there is nearly 100% cell disruption with little cross contamination of nuclei and cytoplasm (unpublished results). The isolation of RNA containing A-rich sequences

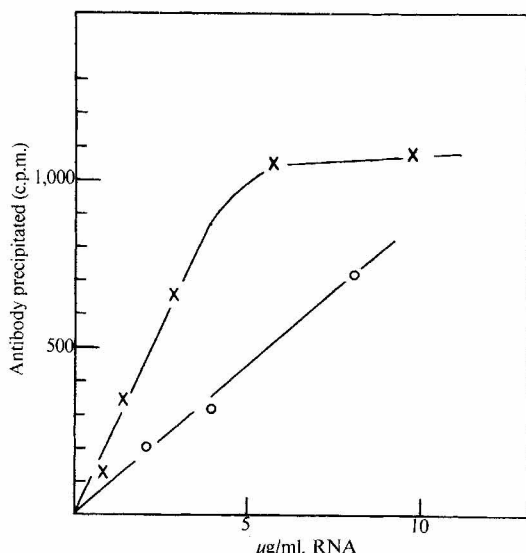
was performed by the phenol extraction method of Lee *et al.*<sup>4</sup>; first at pH 7.6 to remove ribosomal RNA and then at pH 9.0 to remove A-rich RNA. Over 90% of the A-rich RNA is found in the pH 9.0 fraction. RNA containing A-rich sequences was isolated for radioactivity determinations by the 'Millipore' filter technique<sup>4</sup>, in which RNA containing A-rich sequences specifically bind to 'Millipore' filters. For isolation of A-rich RNA for injection into *Xenopus laevis* eggs, a poly-U column was prepared by the method of Britten<sup>11</sup>. Over 90% of the pH 9.0 RNA binds to the column while less than 5% of pH 7.6 extracted RNA binds.

## Size of RNA containing A-rich Sequences

The distribution of radioactive RNA from 5563 myeloma cells, which were labelled with <sup>3</sup>H-adenosine for 1.5 h in the presence of 0.25 µg/ml. actinomycin D and extracted and analysed as above, is shown in Table 1. Over 40% of the total labelled RNA in the cytoplasm contains A-rich regions as judged by binding to 'Millipore' filters. Following digestion with DNAase, and pancreatic and T<sub>1</sub> RNAase<sup>6</sup>, 30% of this



**Fig. 1** Electrophoretic analysis of pH 9.0 RNA fractions from nuclei and cytoplasm. The nuclear (○) and cytoplasmic (●) pH 9.0 RNA from Table 1 were resuspended in electrophoresis buffer (0.04 M Tris, 0.02 M sodium acetate, 2 mM EDTA, 0.5% SDS, pH 7.4) and mixed with <sup>32</sup>P<sub>4</sub>-labelled ribosomal marker RNA. Electrophoresis on 2.6% acrylamide gels and radioactivity determinations were performed as described earlier<sup>13</sup>.



**Fig. 2** 5563 IgG production in *Xenopus* eggs injected with RNA containing sequences from nuclei or cytoplasm of 5563. A poly-U column was prepared by mixing a poly-U solution with 'Geon 425' beads and drying the mixture at 100° C. The poly-U was fixed to the beads by ultraviolet light irradiation. The pH 9.0 RNA was ethanol precipitated and resuspended in 0.1 N NaCl. The solution was passed through the poly-U column (10×1 cm) at 4° C and the column washed with 30 ml. of 0.1 N NaCl at 15° C. The A-rich RNA was eluted from the column with 0.01 M Tris-HCl (pH 7.6) at 37° C. The cytoplasmic and nuclear RNA from the poly-U column were precipitated from two volumes of ethanol, dried and resuspended in 0.4 ml. of injecting buffer (88 mM NaCl, 1.0 mM Tris-HCl, pH 7.6). Then 0.1 ml. was removed and the RNA concentration determined by the absorbance of 260 nm using the relationship  $A_{260} 20 = 1$  mg RNA. To the remaining RNA, an equal volume of injecting buffer containing  $^{14}$ C-amino-acid mixture (2.5 mCi/ml.) was added and five serial 1 : 1 dilutions were made with injecting buffer containing  $^{14}$ C-amino-acid mixture (1.25 mCi/ml.). Injections (150 nl.) of the dilutions were made according to Gurdon *et al.*<sup>14</sup> into eggs obtained from a *Xenopus laevis* female induced to lay eggs by injection 25 h earlier with 500 units chorionic gonadotrophin. Following injection of RNA, each sample of twenty eggs was incubated for 4 h at room temperature. The samples were then homogenized in a 'Dounce' homogenizer and centrifuged at 20,000 r.p.m. for 15 min in the SW40 rotor. Anti-5563 antiserum (5 µl.) was added to each sample (0.25 ml.) of supernatant fluid and incubated at 37° C for 15 min. Goat anti-rabbit antiserum (140 µl.) was then added and the mixture incubated at 37° C for an additional 30 min. After overnight at 4° C, the precipitates were washed three times with cold PBS, dissolved in alkali and the radioactivity determined. The radioactivity of a precipitate control (~300 c.p.m.) has been subtracted from each value. ○, Nuclear RNA; ×, cytoplasmic RNA.

RNA can still bind to the filter, suggesting that a third of the labelled adenosine is in the A-rich region. Labelled RNA from the nucleus contains nearly 20% RNA containing A-rich sequences capable of binding to the 'Millipore' filters, and approximately 10% of this is resistant to nuclease treatment.

When the RNA containing A-rich sequences obtained by the pH 9.0 extraction of the nucleus or cytoplasm is analysed by electrophoresis on 2.6% polyacrylamide gels, the pattern in Fig. 1 is obtained. Over 80% of the cytoplasmic RNA migrates faster than 28S ribosomal marker RNA, while over 60% of the nuclear RNA migrates slower than 28S marker RNA, with a small overlap of the two types of RNA between 28S and 18S RNA. Following RNAase digestion, the A-rich RNA from both the cytoplasm and nucleus show similar electrophoretic patterns, migrating slower than transfer RNA with a suggested size of 150–250 nucleotides (manuscript in preparation). Similar electrophoretic patterns have been obtained in HeLa cells<sup>5,6</sup>.

It is apparent that not all of the nuclear RNA has A-rich sequences attached as has been suggested<sup>5</sup> since only 20% of

the total nuclear radioactivity resides in RNA containing A-rich sequences. Furthermore, this RNA shows a size reduction over the total nuclear RNA, the largest molecules being slightly larger than the 45S rRNA precursor while total nuclear RNA exhibits molecules of considerably greater size (90S)<sup>2</sup>. Whether the sequences containing A-rich RNA are derived from the larger nuclear RNA is not apparent at this time.

The heavy chain and light chain of 5563 immunoglobulin contain approximately 450 and 200 amino-acids, respectively, and their corresponding messenger should contain 1,350 and 600 nucleotide bases. From the previously estimated average size of the A-rich region<sup>6</sup>, and the proportion of  $^3$ H-adenosine counts in the A-rich region (Table 1), the average size of the cytoplasmic and nuclear RNA can be calculated to be 2,400 and 7,200 nucleotides respectively. As the exact base composition of the RNA is not known, a 50% GC content in the non-A-rich regions of the RNA is assumed. These average sizes are consistent with the electrophoretic analysis in Fig. 1. Although the average messenger size in the cytoplasm is large relative to the predicted coding size of the myeloma mRNA, there is a precedent for this in the case of globin message which contains over 70% more nucleotides than are necessary to code for the globin chain<sup>12</sup>.

## 5563 Immunoglobulin produced in *Xenopus* Eggs

The cytoplasmic or nuclear RNA containing A-rich sequences eluted from the poly-U column was resuspended and diluted with injecting buffer containing  $^{14}$ C-amino-acid mixture. Following injection of the RNA, the eggs were incubated for 4 h, homogenized and the specific 5563 radioactive protein was determined by indirect antibody precipitation. The precipitates were allowed to form overnight and the radioactivity determined. The controls consisted of eggs injected with isotope only, and eggs injected with RNA but precipitated with normal rabbit serum instead of anti-5563 antiserum.

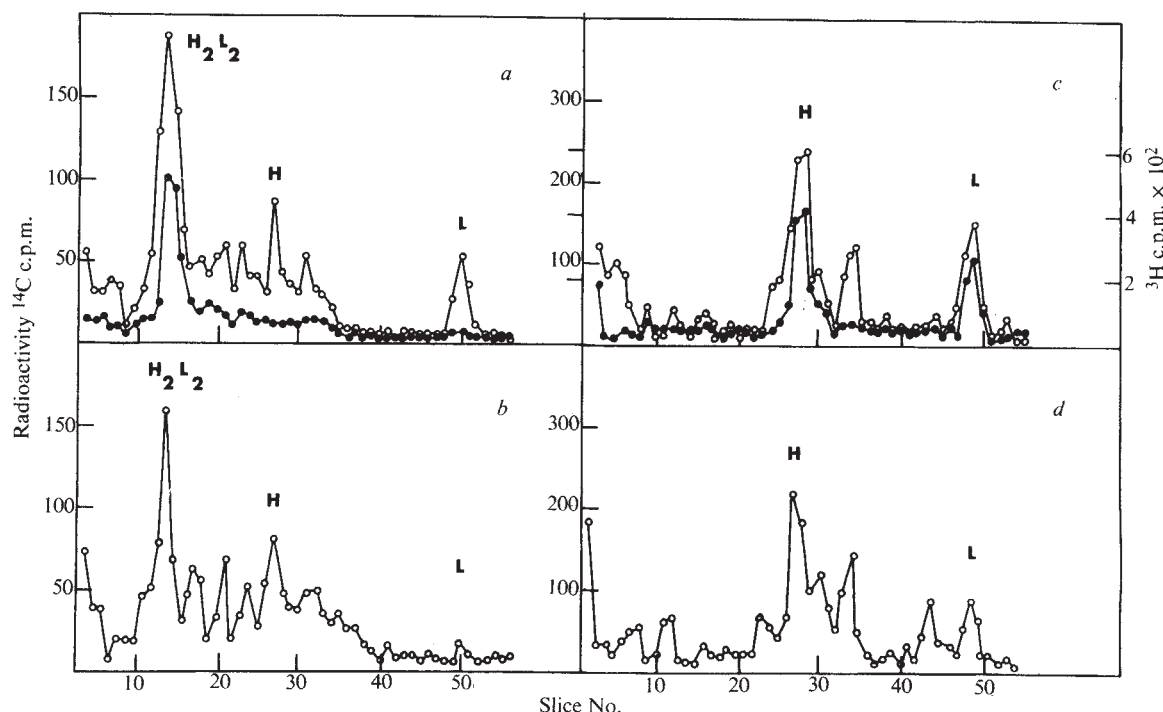
For both cytoplasmic RNA and nuclear RNA, there is an apparent linear response of 5563 immunoglobulin production with increasing amounts of RNA injected (Fig. 2). The cytoplasmic RNA, however, shows a 2.5-fold greater specific activity than the nuclear RNA. This is in close agreement with the three-fold specific activity difference predicted from the calculated average size differences of the RNA in the pH 9.0 fractions of the nucleus and cytoplasm, and from the size differences of the RNA on acrylamide gels. This suggests that both cytoplasmic RNA and nuclear RNA are translated with equal efficiency in this system.

The saturation of the *Xenopus* translation machinery by injected cytoplasmic RNA appears to occur at about 6 µg RNA/ml. (1.5 ng RNA/egg), which is nearly five times lower than the saturating RNA concentration for haemoglobin mRNA in a similar system. The reason for the difference is unknown. The low yield of nuclear RNA has precluded obtaining a saturation curve for it at this time.

## *Xenopus* Oocyte Products

For electrophoretic analysis of the product synthesized in *Xenopus* oocytes, the anti-5563 antibody precipitation of protein from both the nuclear and cytoplasmic RNA injected oocytes was carried out in the presence of  $^3$ H-5563 immunoglobulin, to serve as an internal marker. The antibody precipitates were dissolved in 2.0% SDS and either alkylated with iodoacetamide and analysed on 7.5% acrylamide gels or reduced with mercaptoethanol before subsequent alkylation and electrophoresis. The nuclear and cytoplasmic mRNA fractions yield similar electrophoretic patterns of translation products precipitated by specific antibody (Fig. 3). Gel electrophoretic analysis of the non-reduced samples shows that the major specific product made in the oocytes is completely





**Fig. 3** Electrophoretic analysis of the products precipitable by specific antibody. Specific products of translation of (a) cytoplasmic RNA, and (b) nuclear RNA. Specific products after reduction with 0.1 M mercaptoethanol, (c) cytoplasmic RNA, and (d) nuclear RNA. Samples of thirty oocytes were injected with either cytoplasmic RNA (15 µg/ml.) or nuclear RNA (30 µg/ml.) resuspended in injection buffer containing 1.25 mCi/ml.  $^{14}\text{C}$ -amino-acid mixture. Incubation (6 h) was performed in 66% Hanks<sup>15</sup> BSS containing the above concentrations of  $^{14}\text{C}$ -amino-acid mixture. The eggs were homogenized,  $^3\text{H}$ -5563 protein was added and the indirect antibody precipitation was performed as described under Fig. 2. The washed immunoglobulin precipitates were dissolved in 2% SDS and heated to 100° C for 1 min. A portion of the nuclear and cytoplasmic RNA infected samples was alkylated with iodoacetamide (0.1 M) and loaded on a 7.5% acrylamide gel. Another portion of each sample was reduced by mercaptoethanol (to 0.1 M). Following incubation at room temperature for 1 h at 50% molar excess iodoacetamide was added, the solution heated to 100° C for 1 min and then layered on a 7.5% acrylamide gel. Electrophoresis was performed as described by Summers<sup>16</sup>. ○,  $^{14}\text{C}$ -*Xenopus* produced products; ●,  $^3\text{H}$ -marker 5563 protein produced *in vivo*.

assembled 7S myeloma protein ( $\text{H}_2\text{L}_2$ ) whether the injected RNA was obtained from nucleus or cytoplasm. The  $\text{H}_2\text{L}_2$  peak is obtained whether or not the oocytes are disrupted in 0.1 M iodoacetamide, indicating that the assembly into  $\text{H}_2\text{L}_2$  occurs within the oocyte. In both samples there are also lesser amounts of free heavy and light chains as well as components intermediate in size between 7S immunoglobulin and heavy chains that could tentatively be designated as  $\text{H}_2\text{L}$ ,  $\text{H}_2$  and HL in descending order of size. The electrophoretic pattern

of the reduced samples shows that most of the specifically precipitable counts are in heavy and light chains. The only additional unexplained peak of any significant size is a component slightly smaller than the heavy chain. This peak is present in a similar position in the non-reduced samples and has been detected, though in lesser amounts, in other experiments; the relevance of this component to the specific myeloma products is unknown. Antibody precipitates made in the presence of endogenous products from *Xenopus* eggs or

**Table 1** Quantitation of A-rich Containing RNA

|           | Total RNA *<br>c.p.m. | A-rich containing †<br>RNA<br>c.p.m. | A-rich RNA ‡<br>c.p.m. | % A-rich<br>containing<br>RNA | % A-rich RNA in<br>A-rich containing<br>RNA | % A-rich RNA<br>in total RNA |
|-----------|-----------------------|--------------------------------------|------------------------|-------------------------------|---------------------------------------------|------------------------------|
| Cytoplasm | $9.6 \times 10^4$     | $4.1 \times 10^4$                    | $1.3 \times 10^4$      | 43                            | 32                                          | 13.5                         |
| Nucleus   | $4.0 \times 10^5$     | $7.6 \times 10^4$                    | $8.4 \times 10^3$      | 19                            | 11                                          | 2.1                          |

Myeloma cells were routinely grown at 37° C in L-15 media containing 15% foetal calf serum.  $5 \times 10^6$  myeloma cells were incubated for 15 min at 37° C with 0.5 µg/ml. actinomycin D to suppress ribosomal RNA synthesis.  $^3\text{H}$ -Adenosine (100 µCi/ml.) was added and incubation continued for 1.5 h. The cells were washed twice with PBS (0.14 M NaCl, 0.02 M phosphate buffer, pH 7.4) and resuspended in 2.0 ml. 0.7% NP-40 in PBS. Following incubation at 4° C for 15 min, the mixture was centrifuged at 3,000g for 5 min. The supernate was termed cytoplasm. The nuclei were washed and then resuspended in 2 ml. PBS. Then 15 ml. pH 7.6 buffer (50 mM Tris-HCl, pH 7.6, 50 mM KCl, 1 mM  $\text{MgCl}_2$  and 0.5% sodium dodecyl sulphate (SDS)) was added to the nuclei and cytoplasm. Phenol was added (30 ml.) and the mixtures shaken, centrifuged and the aqueous phase removed. The phenol was re-extracted once with 7.6 buffer and then twice with pH 9.0 buffer (Tris-HCl, pH 9.0). The two 7.6 and 9.0 fractions were individually combined, re-extracted with phenol and the RNA precipitated from the aqueous phase by two volumes of ethanol at -20° C. For nuclease digestion, the RNA was resuspended in TSM (0.100 mM NaCl, 0.10 mM Tris-HCl, pH 7.4, 1 mM  $\text{MgCl}_2$ ) and DNAase (20 µg/ml.) was added. Following 30 min incubation at 37° C EDTA (final concentration) pancreatic RNAase (2 µg/ml.) and T<sub>1</sub>RNAase (2 units/ml.) were added and the incubation continued for 30 min. The mixtures were diluted ten-fold with high ionic strength buffer (10 mM Tris-HCl, pH 7.6, 500 mM KCl, 1 mM  $\text{MgCl}_2$ ) at 4° C and collected on 'Millipore' filters (Type HA, 0.45 µ) for radioactivity determinations.

\* Total = trichloroacetic acid (TCA) precipitate of pH 7.6 and 9.0 RNA.

† RNA bound to 'Millipore' filter at high ionic strength.

‡ RNA bound to 'Millipore' filter at high ionic strength following nuclease digestion.

oocytes injected only with radioactive amino-acids showed only background levels of radioactivity when analysed on acrylamide gels.

The most noticeable difference between the translation products of nuclear and cytoplasmic mRNA is the deficit of free light chain relative to free heavy chain produced from nuclear mRNA. This deficit is seen in both the unreduced and the reduced immunoglobulin samples. The light and heavy chain ratio from cytoplasmic mRNA translation in the oocyte is in agreement with the observed ratio in the cultured 5563 myeloma cell. The apparent imbalance of light and heavy chain mRNA in the nucleus suggests that there could be a defect in mRNA transport from the nucleus.

The fact that the major specific product in the oocyte is the assembled  $H_2L_2$  molecule is most striking. Vassalli, Lisowska-Bernstein and Lamm<sup>17</sup>, studying the cell-free synthesis of rat immunoglobulin, showed that, using microsomal vesicles as the polyribosome source, one of the major products inside the vesicles was  $H_2L_2$ ; if, however, the polyribosomes were released from membranes before cell-free incubation, the heavy and light chains synthesized were released free into the medium and were not further assembled. Since immunoglobulin chains are vectorially released<sup>18</sup> through the membranes binding the polyribosomes, the results of the cell-free system suggest that the microenvironment of the cisternal space is important for assembly. It will be intriguing to ascertain what feature of the oocyte permits such efficient assembly of  $H_2L_2$  from heavy and light chains made therein.

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# Quaternary Structure of Gastropod Haemocyanin

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**Three-dimensional image reconstruction shows haemocyanin to be made of three distinct parts. At the ends of the particle are a five-fold "collar" and a central "cap". The cylindrical wall consists of sixty dimers which may be correlated with 120 oxygen binding sites.**

THE use of negative staining and of new methods of image analysis have greatly advanced the study of biological assemblies by electron microscopy<sup>1</sup>. We describe here an investigation which has given a clear picture of the quaternary structure of haemocyanin. Although the ultimate subunits have not been resolved, the symmetry relations between them can be deduced from the packing of the morphological units revealed by the analysis.

Haemocyanins are respiratory proteins containing copper and which occur freely dissolved in the haemolymph of many

invertebrates<sup>2</sup>. The reaction with oxygen is reversible, one molecule of  $O_2$  being bound per two copper atoms. The gastropods contain the largest known haemocyanins, of molecular weight about  $7-8 \times 10^6$ . These undergo a series of association-dissociation reactions, depending on pH and ionic strength, first observed with the ultracentrifuge<sup>3,4</sup>.

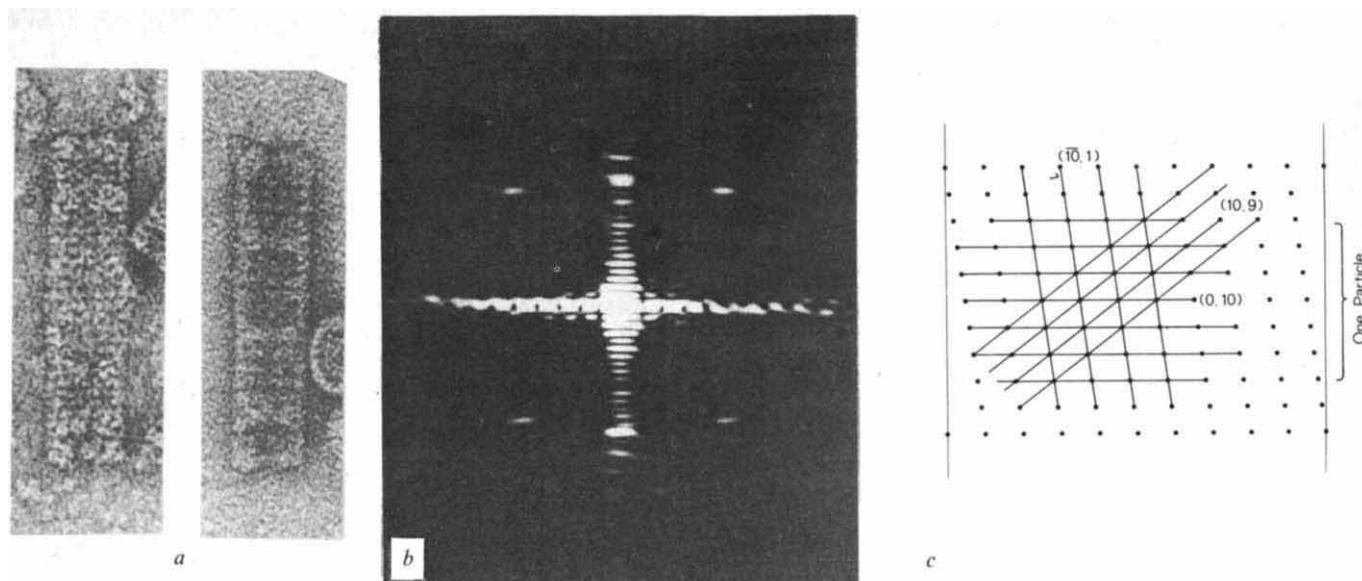
Electron microscopy of gastropod haemocyanin has shown that the particles are cylindrical and that the first dissociation step into halves occurs in a plane perpendicular to the cylindrical axis<sup>5,6</sup>. The images observed have not been related to a three-dimensional structure in a quantitative way, but the arrangement of subunits has been reported to have five-fold<sup>5</sup> or ten-fold<sup>6</sup> symmetry. We have studied haemocyanin from *Kelletia kelletia* (a sea snail), *Busycon canaliculatum* (a whelk) and *Helix pomatia* (vineyard snail). We have found no fundamental difference in structure, and the variations detected seem to reflect differences in the preparations.

## General Observations

A field of negatively stained haemocyanin particles generally displays two characteristic views<sup>5,6</sup>. A rectangular image, corresponding to the side-on view of the cylinder of 360 Å length, shows a characteristic staining pattern of striations

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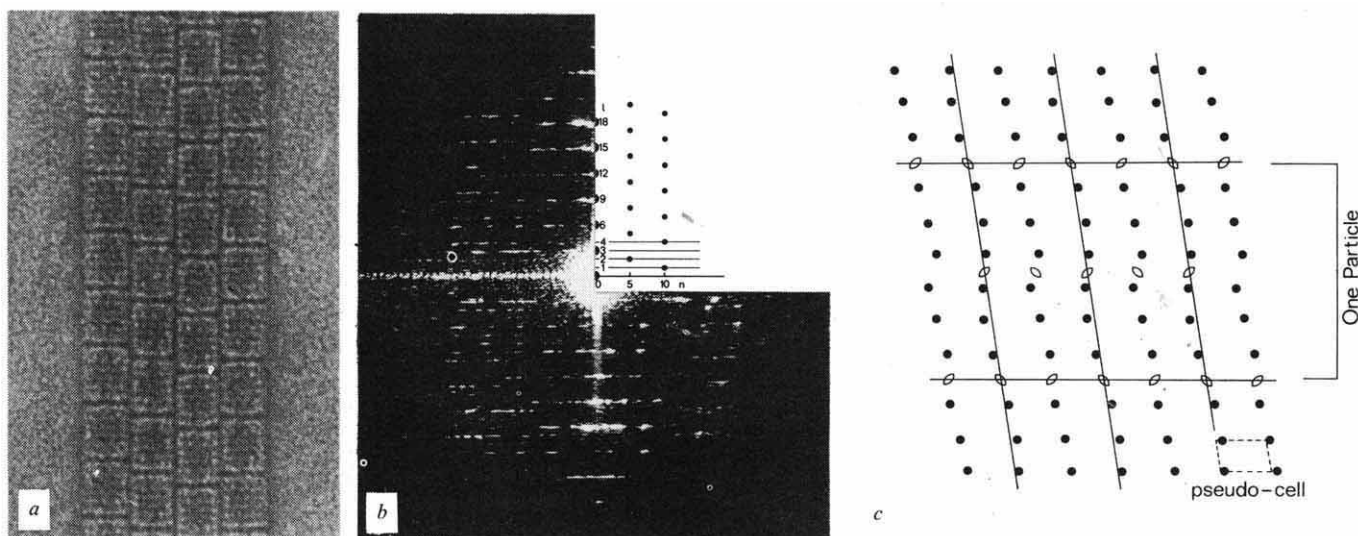




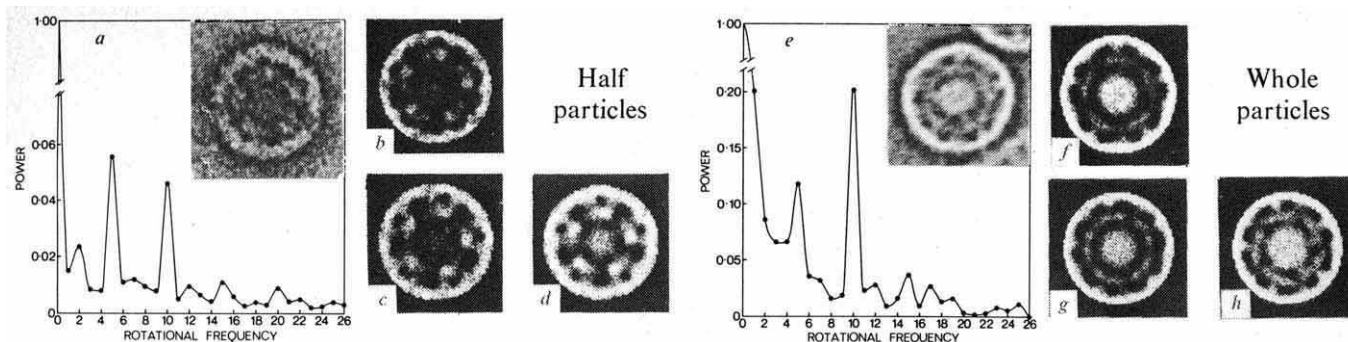
**Fig. 1** *a*, Electron micrographs of linear polymers of *Busycon haemocyanin*. Polymers were obtained by leaving haemocyanin solutions of low ionic strength to stand at 4° C in a range of pH about the isoelectric point of 4.4. The polymer on the right was photographed in uranyl stain over a hole in a carbon foil, that on the left on the carbon foil. The particles are flattened compared with those in the middle rows of Fig. 2*a*. *b*, Optical diffraction pattern of the polymer shown at the right in *a*. The strong meridional reflexion is at an axial spacing of 60 Å, and the non-meridional reflexion at 67 Å, corresponding to layer lines 9 and 10 of an axial repeat of 600 Å. In stronger exposures the second orders of the two main reflexions are visible. The helical selection rule for the pattern is  $l = n + 10m$ , the only permitted values of  $n$  being multiples of 10. *c*, The surface lattice, with three helical families marked (although the grooves of the family (10, 1) are seldom observed). The unit cell defined by (0, 10) and (10, 9) has an axial height of 60 Å and a width of 80 Å at a radius of 140 Å. The sense of (10, 9) was chosen to be right-handed so as to be the same as that found by tilting in *Kelletia haemocyanin*.

oriented perpendicular to the cylindrical axis (Figs. 1 and 2). There are five such striations on a particle, suggesting that the corrugated cylindrical wall is made up of six rings or layers of subunits. A circular end-on view shows an outside ring of external diameter 300 Å with a characteristic pattern inside (Fig. 3). The material present at the centre is rather variable and sometimes seems to detach from the particle.

The end-on views can be analysed in two dimensions to give the rotational symmetry of the particle about the cylinder axis. To obtain a three-dimensional image of the particle, however, other projections must be used. It has been shown<sup>7</sup> that a quantitative reconstruction of a structure can be made from a number of projections covering a viewing range of about 180°. If an object has helical symmetry, a single image may



**Fig. 2** *a*, An array of laterally aggregated linear polymers of *Kelletia kelletia haemocyanin*. This "complex" was prepared by allowing the haemocyanin to stand in 0.001 M NaAc buffer at pH 4.5 for a few days at 4° C. The stain is uranyl acetate and the particles are supported on a carbon film. The middle rows have a smaller width than the outer ones which are flattened. *b*, Optical transform of *a*. The pattern shown here is affected by interference of neighbouring polymers in the array which shows up as modulations in intensity along the layer lines, but this does not affect the interpretation. The order in the micrograph of these specimens extends to axial spacings of about 30 Å (visible in stronger exposures). The off-meridional reflexions extend to about  $1/50 \text{ Å}^{-1}$ . The layer lines are approximately equally spaced at orders of an approximate repeat of 1125 Å. The meridional peaks on layer lines 3, 6, 9, 12 and so on arise from the spacing of 375 Å between particles. The strongest is at  $l=18$ , corresponding to a spacing of 60 Å, the distance between layers in a particle. In most transforms only one Bessel function contributes to each layer line. On layer lines  $l=3m$ ,  $J_0$  terms are present.  $J_{5s}$  occur on layer lines 4, 7 and 10 and  $J_{10s}$  on layer lines 2, 13, 16 and 19. Inset, top right: the  $n-l$  plot for the diffraction pattern. The selection rule is  $l = n + 3m$ , with permitted values of  $n$  which are multiples of five. The absolute hand of the structure was determined by analysing the phase change on the strong layer lines of transforms of tilted polymers. *c*, Part of the surface lattice deduced from *b*. The unit cells are shown outlined. The presence of two-fold axes, and the existence of a smaller pseudo-cell, is justified in the text. Note the correspondence with the *Busycon* lattice in Fig. 1*c*.



**Fig. 3** Analysis of rotational symmetry of whole and half particles. Negatively stained specimens were made of *Busycon* haemocyanin solutions at pH 4.0 and 6.9 (in 0.01 M Na acetate). At pH 6.9 the particles are known to be whole, whereas at pH 4.0 half particles exist in solution<sup>3</sup>. [Particles were embedded in a sheet of uranyl acetate stain over holes in a carbon foil. The difference between whole and half particles can be checked by the presence of a much more intense modulation in contrast at the outside of whole particles compared with half particles. This is due to accumulation of stain at the outside surface along the direction of projection. When situated on a carbon foil the particles were, as a rule, not so well preserved. In fields of shallow stain, the end-on views of whole particles tend to have a strong five-fold appearance. The most likely explanation for this is that the particles are not uniformly embedded in the stain so that they are probably more one-sided than those over holes]. The results shown here are typical examples of an analysis of about ten end-on views for each case. The first criterion for selecting the views from the micrographs during the analysis was the presence of a circular outer contour, as most of the end-on views tended to be misaligned with respect to the beam or were not well preserved. The results of the analysis are expressed in a power spectrum, compare *a* and *e*, which measures the total weight of each  $n$ -fold component in an image<sup>10</sup>. It is clear that in both whole and half molecules the reliable rotational frequencies present are multiples of five. In the best examples the rotational order is preserved to spacings of about 25 Å. Up to and including the rotational frequency twenty, the ratio of the power in the four five-fold harmonics to that in all other sixteen azimuthal components is about 0.46 in case *a* and 0.53 in case *e*. The proportion of the total power in the circularly symmetric component  $n=0$  plus the five-fold components is 0.90 and 0.94 respectively. Rotationally filtered images (*b-d* and *f-h*) were calculated by a Fourier synthesis in which only five-fold harmonics and the circularly symmetric term were present. The filtered images *b* and *f* derive from the originals shown in insets *a* and *e* respectively.

provide these projections and allow a three-dimensional reconstruction of the density, at least to a limited resolution. The point group symmetry of a single particle of haemocyanin is, as we shall show,  $D_5 : 52$ , and this does not provide enough independent projections to solve the structure to better than 100 Å. We therefore took advantage of the known tendency of some haemocyanins to aggregate end-to-end to form linear polymers<sup>8,9</sup> and studied these in the hope that the screw angle between particles in the polymer would be favourable for a helical reconstruction.

### Analysis of End-on Views

A method for the quantitative harmonic analysis of end-on views of particles with rotational symmetry has been described by Crowther and Amos<sup>10</sup>. The image is digitized and computationally decomposed into cylindrical waves, and the symmetry determined by assessing the relative strengths of the  $n$ -fold symmetry components present, as expressed in a power spectrum. In a second step, those components which satisfy the symmetry found are combined to produce a filtered image.

The method was applied to end-on views of whole and half particles of haemocyanin (Fig. 3). The strongest components in the rotational power spectra of both whole and half particles are all multiples of five. The filtered image of the half-particle shows a strong five-fold modulation in density situated about halfway from the centre to the edge of the particle, whereas the outer part is dominated by a ten-fold modulation. On the other hand, in the end-on view of a whole particle the central five-fold modulation, though still present, is considerably reduced in strength in comparison with the half molecules. The outer part remains ten-fold in character.

An explanation of the reduction of the strength of the five-fold component on going from half to whole molecules is that the two halves in a molecule are not in perfect rotational register, but are staggered. This is confirmed by our reconstruction of whole molecules described below, which shows that the two halves are rotated relative to each other by about 6°.

### Arrays of Molecules

We have found conditions for preparing linear aggregates of *Busycon* and *Helix* haemocyanin. They often associate side by side to form a two-dimensional array, but the order in

these arrays is not crystalline in the lateral direction. A typical polymer of *Busycon* haemocyanin is shown in Fig. 1, together with its optical transform. The strong meridional reflexion is at a spacing of 60 Å, the distance between layers in the particle. The distance of the strong off-meridional maximum from the meridian is consistent with a ten-fold variation in density at a radius of about 150 Å, and correlates with the ten-fold modulation seen in the outer region of the end-on views of the particles. The surface lattice corresponding to the two main diffraction maxima is shown in Fig. 1c. There are sixty unit cells in one particle, ten in each of the six layers. The relative orientation of the successive particles in the linear polymer is such that the surface lattice continues without interruption.

A similar kind of array can be prepared from *Kellettia* haemocyanin<sup>8</sup> (Fig. 2). The transform of the image shows a set of layer lines typical of a helical arrangement with a long axial repeat distance. This is about 1150 Å which corresponds to the length of three particles, successive particles being rotated by 120°. The asymmetric unit is a sector of one particle with an angular width of  $2\pi/5$  (Fig. 2c). There are fifteen independent projections of this asymmetric unit in the repeat distance of the polymer, which is therefore suitable for a three-dimensional reconstruction.

A preliminary search was made over a large number of "sandwiched" polymers in arrays of *Kellettia* haemocyanin for those with clear optical transforms, having good symmetry about the meridian. A further selection was made on the basis of the phase correlation on the layer lines of the computed transform<sup>7</sup>. The state of preservation of the particles in an array was tested by tilting experiments. In many cases the width of one row of "sandwiched" particles hardly changes when tilted about the cylinder axis by 72°, nor do the radial positions of the off-meridional spots in the corresponding optical transform. This indicates that there is little flattening, and the good preservation can be confirmed by following the variation of the phases of these Fourier components on tilting. The state of preservation of isolated linear polymers, like those of single particles lying side-on on the grid, is never as good as those within an array.

The search finally gave four linear polymers in different arrays which showed a good phase correlation (within 30°) on the layer lines with the strongest reflexions. From each of these polymers, a length of two repeats, comprising six



particles, was used to calculate the Fourier components for a three-dimensional synthesis<sup>11</sup>. The four reconstructions were very similar to one another, and an average of the four was calculated after the relative orientations of the individual polymers had been determined by comparing the relative phases in the transforms. In all the reconstructions there was a strong indication that the two halves of the particle on either side of the equator were related by a set of two-fold axes lying perpendicular to the cylindrical axis. The associated two-fold axes in the plane separating two neighbouring particles were also strongly indicated. The presence of dyads was therefore tested for directly in the original transform of each array by searching for the position of putative dyads which make the phases on each layer line as close as possible to  $0^\circ$  or  $180^\circ$  (using an unpublished program of L. Amos). The lowest values of the residuals found were respectively  $26^\circ$ ,  $28^\circ$ ,  $30^\circ$  and  $32^\circ$  for the individual polymers, and  $19^\circ$  for the average, leaving little doubt that horizontal dyads are present to the resolution of the micrographs.

We therefore imposed a two-fold symmetry on the data by setting the phases of the averaged layer line data to  $0^\circ$  or  $180^\circ$ , depending on the original value. The final map, comprising the average data from forty-eight half-particles, has a resolution of about  $30 \text{ \AA}$  in the axial direction and  $50 \text{ \AA}$  in the radial direction.

## Description of the Structure

The structure calculated from the averaged data from all four polymers with imposed two-fold symmetry is presented in Figs. 4, 5 and 6. The cylindrically averaged map (Fig. 4) and wooden model (Fig. 5) show the gross morphology of the particle. The molecule is like a hollow cylindrical drum closed at the ends. The end consists of the five-fold material which we call the "collar" and the material at the centre which we call the "cap".

Fig. 6 shows cylindrical sections from the three-dimensional map, superimposed in pairs, starting at a radius of  $40 \text{ \AA}$  up to the maximum radius of about  $150 \text{ \AA}$ . This representation is convenient for following both the detailed structure of a

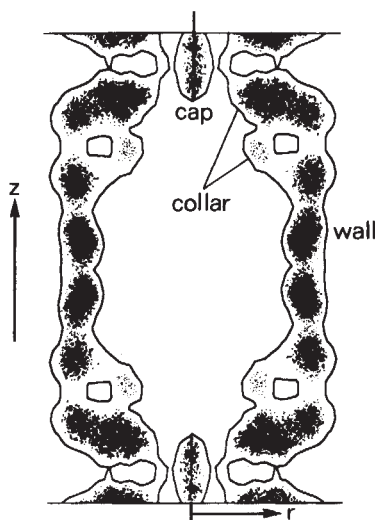


Fig. 4 The cylindrically averaged density distribution<sup>15</sup> of *Kelletia* haemocyanin calculated by a Fourier synthesis using only the  $J_0$  terms on the layer lines  $l=3$  m, up to  $l=45$  ( $0.04 \text{ \AA}^{-1}$ ). The data were an average from four different polymers, as described in the text. Only one contour line, just above the zero level, is drawn to show the positive density in the map. The highest densities are stippled. (Not shown in the map are some small peaks close to the cylindrical axis, at which special position errors tend to build up. In the individual reconstructions of the four polymers these peaks are quite variable.) The main parts of the structure are named. The wall is involuted at the equator, because of the inward tilting of the two middle rings.

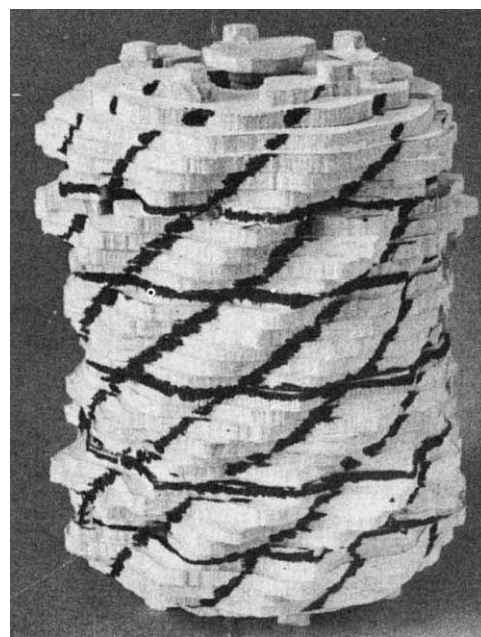


Fig. 5 A wooden model of the haemocyanin particle. This model was built from a series of axial sections in the three-dimensional Fourier density map. The morphological units of the wall are outlined.

single particle and the relation between neighbouring particles in the polymer. Neighbours in the polymer make contacts at a radius of about  $90 \text{ \AA}$  and also in the region of the cylinder axis (shown in Fig. 4).

The cylindrical wall of the particle has approximately ten-fold rotational symmetry and consists of sixty morphological units, which begin in the section at  $80 \text{ \AA}$ , and are at their widest at about  $120 \text{ \AA}$ . These units are bounded by three dominant sets of grooves made visible by the stain modulations. One set runs perpendicular to the cylinder axis giving rise to the horizontal striations visible in the original images. The other sets each consist of ten lines running in approximately the same directions as the two oblique sets of helical lattice lines of the *Busycon* polymer (Fig. 1c). These lines divide the wall into pseudo-cells (Fig. 2c), but the division does not constitute a strict surface lattice because the lines are not straight (Fig. 6) but appear to be "sinusoidally" perturbed versions of those present in the *Busycon* polymer. The pseudo-lattices of successive particles in the *Kelletia* polymer are approximately in register, and the oblique grooves run more or less continuously across the inter-particle boundaries.

The material within the walls of the particle, that is, within a radius of  $110 \text{ \AA}$ , has a clear five-fold character, which justifies its being named as a distinct component—the collar. It extends to a radius of about  $35 \text{ \AA}$ . In the axial direction this five-fold material extends from the end of a particle to the second ring.

## Functional Implications

The three-dimensional structure confirms the inference from the dissociation behaviour that the haemocyanin particle consists of two identical halves and shows that these are related by two-fold axes in the equatorial plane. There are three distinct parts, the outer wall, comprising the bulk of the protein, and the minor structures which we have called the collar and the cap. The wall consists of sixty morphological units, which are of six crystallographically distinct types (two in each layer of the half particle). But they are of similar size, shape and orientation, and appear to be quasi-equivalent. We have already remarked that the surface lattice found in the polymers from *Kelletia* is a perturbed version of that in *Busycon*, in which the

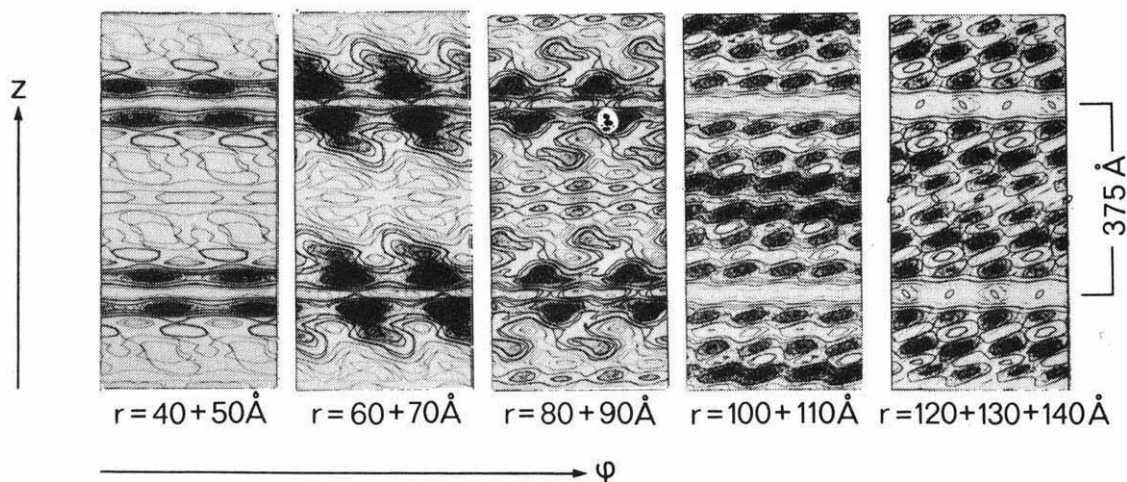


Fig. 6 Three-dimensional reconstruction of polymers of *Keltelia* haemocyanin. Cylindrical sections, of angular width  $2/5 \times 2\pi$ , at adjacent radii are superimposed. The data used are averaged from four different polymers. The shaded areas represent the higher densities of protein. The positions of the two-fold axes in the point group 52 are marked. [The equatorial data are sensitive to departures from cylindrical symmetry in the distribution of the stain which do not, however, affect the other layer lines. The radial distributions obtained by Fourier inversion of the equatorial data of the individual polymers were therefore compared with that found from the Fourier analysis of the end-on views. Three out of the four polymers gave good agreement with the latter, and these were averaged and included in the final three-dimensional summation.]

sixty units of the wall appear identical. We attribute this difference to the fact that the particles in the polymers from *Busycon* are more closely fused end-to-end than in the case of *Keltelia*. This suggests that something has been lost from the "collar" and/or "cap" of the *Busycon* haemocyanin to permit the subunits in the polymer to form a simple lattice which continues without interruption across the inter-particle boundaries.

We conclude therefore that the cylindrical wall of gastropod haemocyanin consists of sixty equal units. In *Keltelia* haemocyanin these units, especially their outer parts, move from the simple lattice positions, producing a sinusoidal perturbation which is predominantly normal to the  $(10, 1)$  helical directions of Fig. 1c. This perturbation is correlated with the integrity of the cap through which the interlocking occurs. We cannot say which lattice would be found for haemocyanin in solution; in other words, whether the natural lattice is the simple one described for *Busycon* or the more complex version of it found for *Keltelia*.

In the *Keltelia* lattice there are strict two-fold axes in the equatorial plane of contact between two half particles and also between neighbouring particles. The relative rotations of the parts of morphological units at a radius of 90 Å on either side of the equator and of the parts at a radius of 100 Å on either side of the interparticle boundary are similar to each other and also to the relative rotation of successive layers of units within a half particle. In other words, successive layers within and between the particles are all rotated through nearly the same angle in *Keltelia* and exactly so in *Busycon*. This suggests that each layer of the wall in the *Keltelia* particle contains ten nearly equivalent dyads rather than merely five strict dyads, so that the point-group symmetry is approximately  $D_{10} : 10, 22$ . This in turn implies that halfway between these dyads there are secondary dyads coinciding with the centres of the morphological units. Thus we infer that these units are themselves dimers, with two-fold axes passing through them in a radial direction. The resolution of detail within the unit is too low to display this dyad, but the overall shape is quite consistent with its presence.

If the local symmetry has been inferred correctly, then the wall of particle is built from 120 "structural" subunits. Assuming a particle weight of about  $8 \times 10^6$ , that is twice the value found for three-ring haemocyanins by van Holde<sup>4</sup>, and ascribing 90% of this material to the wall protein(s), we obtain a molecular weight of about 60,000 daltons for each of the presumed 120 structural units. From the known copper content of the particle

and the fact that two copper atoms are involved in the binding of an  $O_2$  molecule, the number of  $O_2$  binding sites per particle is calculated to be about 130 (compare ref. 12). This strongly suggests that the structural unit of molecular weight 60,000 in the wall can be identified with the functional unit which binds one  $O_2$  molecule. In the particle these 120 "functional" units are arranged in pairs to form sixty dimers.

Finally, one may comment on the possible role of the cap and the collar. The five-fold symmetry of the half particle is due to the presence of the five-fold collar at one end. If the wall of the half particle is built of nonpolar dimers, its polarity must be imposed by the collar and cap. It is possible that the interaction of the collar with the wall perturbs the bonding between layers leading to a quasi-periodic linear aggregate of limited length<sup>13</sup>. One might expect that the collar and cap play a crucial role in the assembly of the particle, perhaps in an analogous way to that in which the base plate of T-even phage acts as a nucleus for the assembly of the whole tail<sup>14</sup>.

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# LETTERS TO NATURE

## PHYSICAL SCIENCES

### Polarization of Extensive Air Shower Emission at 6 MHz

MEASUREMENTS of radio pulses from cosmic ray air showers in the frequency range 20 to 70 MHz have established the dominance of the geomagnetic mechanism<sup>1-4</sup> and there is strong evidence from these experiments that the radio field strength increases with decreasing frequency. There have, however, been few observations at frequencies below 15 MHz where the background noise is considerably higher. At 2 MHz Allan *et al.*<sup>5</sup> found a field strength of  $\sim 750 \mu\text{V/m/MHz}$  for showers with a threshold primary energy of  $10^{17}$  eV. At the same frequency Stubbs<sup>6</sup> found a field strength of  $\sim 0.8 \mu\text{V/m/MHz}$  for a threshold energy of  $2 \times 10^{14}$  eV. Hough *et al.*<sup>7</sup> reported measurements at 3.6 MHz and compared their results with those of Allan *et al.* and Stubbs by normalizing all measurements to those expected on the axis of a shower of primary energy  $10^{17}$  eV and incident normal to the Earth's magnetic field.

Observations of polarization can be used to test the theories of origin. The geomagnetic mechanism would produce a field polarized magnetic east-west. The polarization measurements reported here were made with the Buckland Park aerial array of the University of Adelaide<sup>8</sup>. The array consists of two sets of 89 dipoles arranged on a square (91 m spacing) lattice, and with an overall diameter of about 1 km. One set of dipoles is aligned approximately north-south (actually  $11^\circ$  west of geomagnetic north), and the other set is orthogonal to this.

Although the array was primarily intended to be operated at 2 MHz, it can also be operated on its third harmonic. In the present work, observations were made with both sets of aerials, each connected to form a broadside array, producing zenith pointing beams  $\pm 1.3^\circ$  wide (3 db points) polarized approximately N-S and E-W. The radio receiver had a linear detector and was tuned to a frequency of 5.96 MHz with a bandwidth of  $\pm 40$  KHz.

A pair of vertically-pointing optical Čerenkov detectors, operated in coincidence, was used to detect the extensive air

showers and to trigger a 10 channel counter which recorded the output voltage of the radio receiver. This equipment has been described previously by Stubbs<sup>6</sup>. The resulting record was the mean output voltage of the receiver during ten intervals each of 6.6  $\mu\text{s}$ , and averaged over a number of events. The threshold primary energy of the showers was determined from the average rate of occurrence and the parameters of the optical system. The field of view of this system was similar to the beamwidth of the aerial array. The lateral spread of the Čerenkov light is less than 200 m (ref. 9), so the axes of the observed showers were close to the array centre (within a few wavelengths).

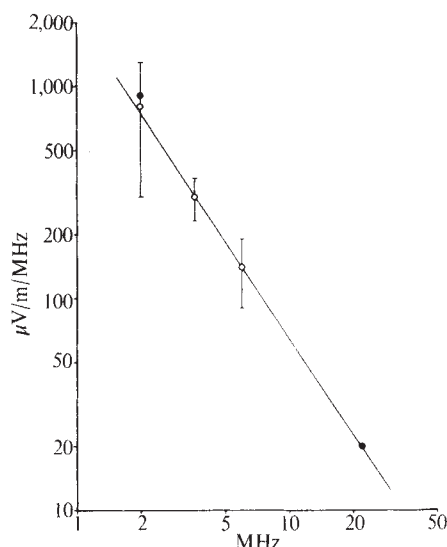


Fig. 2 The normalized frequency spectrum of radio pulses from  $10^{17}$  eV extensive air showers.  $\circ$ , 2 MHz (Stubbs<sup>6</sup>), 3.6 MHz (Hough *et al.*<sup>7</sup>), 6 MHz, present work;  $\bullet$ , 2 MHz (Allan *et al.*<sup>5</sup>), 22 MHz (Prescott *et al.*<sup>12</sup>). Both scales are logarithmic.

On all clear moonless nights between July 2 and July 30, 1971, measurements were made using the north-south aerial, and from July 31 to October 24, 1971, using the east-west aerial. In all, 210 events were studied. Fig. 1 shows the input voltage to the receiver, (a) average over 108 events using the E-W aerial, and (b) averaged over 102 events using the N-S aerial. The continuous line on each figure is the known receiver response to a short fast rising pulse, scaled for ease of comparison. The most significant feature is the delay in response due to the bandwidth of the receiving system. The average height of the radio pulse above the mean noise level was 5.0  $\mu\text{V}$  in the east-west case and 2.2  $\mu\text{V}$  in the north-south case.

Using the 10 channel counter avoids bias in the selection of individual events as only the average result is recorded, but it has a serious disadvantage when using a linear detector in that the noise cannot be subtracted quadratically before averaging. It is impossible to correct fully for this without a knowledge of the radio pulse height spectrum, but a working correction factor was determined experimentally. Bandwidth limited pulses of

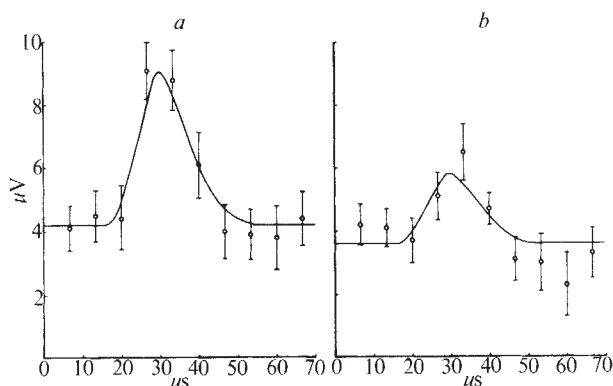


Fig. 1 The average input voltage to the receiver using (a) the E-W aerial and (b) the N-S aerial. The continuous curves show the receiver response to a short pulse applied at  $t=0$  with the height scaled for comparison.

various known heights were combined with noise from the aerial and the average apparent pulse height was measured after the addition of a number of pulses. Hence the ratio of the apparent height in the presence of noise to the true height was determined as a function of signal to noise ratio.

The field strength was determined by calculating the gain of the array assuming a loss free system and no mutual coupling<sup>10</sup> between elements. Any errors introduced by these assumptions would cause the final result to be less than the true value. The E-W aerial gave a field strength of  $0.8 \pm 0.2 \mu\text{V/m/MHz}$  and the N-S aerial  $0.5 \pm 0.2 \mu\text{V/m/MHz}$  for showers with a primary threshold energy of  $10^{15}$  eV. The error is the sample standard error. The presence of an appreciable N-S component suggests that the radio pulse may not be entirely geomagnetic in origin.

It has been shown<sup>11</sup> that the field strength due to the geomagnetic mechanism is proportional to the sine of the angle between the shower axis and the magnetic field. The present results were assumed to be due to a geomagnetic component polarized E-W and a non-geomagnetic component whose amplitude was the same on both aerials. The geomagnetic component was normalized to the value expected when the shower axis is orthogonal to the magnetic field. This analysis yielded a contribution of non-geomagnetic origin which was 34% of the total field. Most workers have found qualitative support for the geomagnetic mechanism but some evidence for non-geomagnetic components has been reported. Prescott *et al.*<sup>12</sup> found a 15% non-geomagnetic contribution at 22 MHz whilst Allan *et al.*<sup>4</sup> found approximately 5% of individual showers were not polarized as expected for a geomagnetic origin. In a statistical analysis Vernov *et al.*<sup>13</sup> found the geomagnetic mechanism to be dominant but not unique.

For comparison with previously reported results the field strength must be further normalized to the same primary energy; this yields a value of  $140 \pm 50 \mu\text{V/m/MHz}$  for showers of primary energy  $10^{17}$  eV. We emphasize that there is still considerable doubt as to the energy dependence of the radio field strength and in our normalization we have assumed that the dependence is essentially linear<sup>11,14</sup>. Fig. 2 compares the above field strength with those of previous experimenters as quoted by Hough *et al.*<sup>7</sup>.

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## Short Period Seismic Discrimination

HERE I describe a method of utilizing the recognized differences in the short period (SP) spectra of earthquakes and underground explosions to aid in their discrimination. The method produces a dramatic increase in the separation between a suite of Nevada Test Site (NTS) explosion events and earthquakes in the Gulf of California area over that obtainable by the TMF (Third Moment of Frequency) method<sup>1,2</sup>.

The TMF discriminant proposed by Weichert<sup>1</sup> exploits the fact that the decay of spectral energy tends to be slower for underground explosions than for earthquakes at frequencies above about 1.5 Hz. Also, several authors have pointed out that explosion spectra decay more rapidly than earthquake spectra at frequencies below about 1 Hz (refs. 3, 4). Fig. 1 shows the average spectra for 49 NTS underground nuclear explosions and 42 Gulf of California (GULF) earthquakes as recorded at the Yellowknife SP array<sup>5</sup> in northern Canada. The spectral differences noted above are quite apparent in Fig. 1; Israelson shows similar curves<sup>6</sup>. The exact shape of the curves will, of course, depend upon the instrumentation used, and in the work described here no attempt has been made to optimize the spectra by instrumental means or by correcting for instrumentation. The spectra of Fig. 1 and all others obtained in this study were derived from the optimum array beam, carefully noise corrected in the manner described by Anglin<sup>2</sup> and normalized, and all events having an *S/N* less than 2 were discarded.

For two populations of spectra that produce overlapping but different average spectra such as those of Fig. 1, it is possible to derive a discriminant that enables the best statistical separation of the populations to be obtained from the individual spectra. As new events are added, however, the nature of the populations (and the optimum discriminant) may change to some degree. In order to avoid continual updating of the algorithm used in evaluating the discriminant it is preferable to settle on one simple algorithm and use it unchanged until a better one is derived. One such is the TMF algorithm which leads to the TMF against complexity discriminant<sup>2</sup> and a preliminary test of another discriminant (MMF against body-wave magnitude) is described here. After sufficient data have been examined, probabilities with regard to correct discrimination of new events may be assigned.

In order to exploit the differences at both ends of the spectrum, I used the discriminant described below, which shows

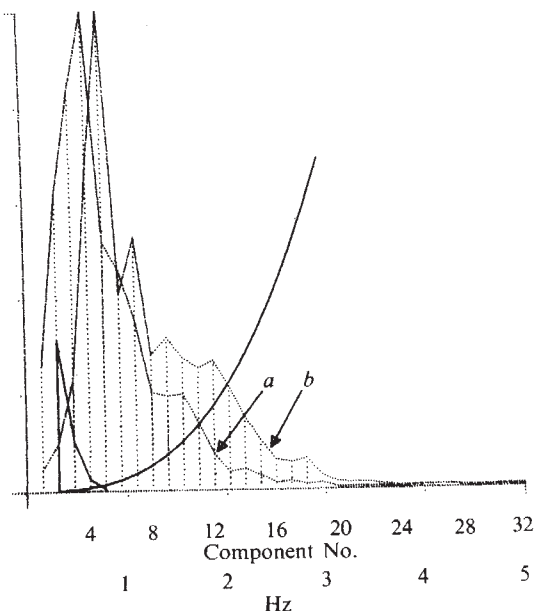


Fig. 1 Average line spectra of 42 GULF earthquakes (a) and 49 NTS explosions (b) with fifth and third moment multiplier curves superimposed to left and right, respectively.



promise although more data are needed, and no doubt the application of the particular algorithm will change in detail. A normal moment of the amplitude spectrum of each event about zero frequency is computed covering the spectrum between arbitrary limits; from this a moment computation operating in the reverse direction from an arbitrary point in the spectrum is subtracted. In this way maximum effect is derived from the upper and lower portions of the spectra where the differences are greatest, and the central portion, where the spectra of explosions and earthquakes are most alike, contributes little. The central portion also tends to be quite unstable in any given population and too much attention paid to it can increase the scatter of the population.

The modified moment of frequency algorithm (MMF) examined is

$$\text{MMF} = \left( \frac{\sum_{k=r}^s (A(k) * k^J)}{\sum_r^s A(k)} \right)^{\frac{1}{J}} - \left( \frac{\sum_{k=p}^q (A(k) * (q-k+p)^M)}{\sum_p^q A(k)} \right)^{\frac{1}{M}} \quad (1)$$

where  $A(k)$  is the relative amplitude of the  $k$ th component;  $J, M$  are the orders of the moments being considered in their respective terms;  $r, p$  are the lowest frequencies (components) considered in their respective terms; and  $s, q$  are the highest frequencies (components) considered in their respective terms. This algorithm only becomes a discriminant when plotted against bodywave magnitude. In Fig. 1 the sharply rising curves superimposed on the two ends of the spectra show the effects of the multipliers ( $k^J$  and  $(q-k+p)^M$ ) for  $J=3, M=5, r=p=2, s=19, q=5$ , which are the values that I found empirically to give the best results of many combinations tried. The spectral components are 0.156 Hz apart, so the above algorithm may be interpreted as follows: the third moment of frequency was computed using components No. 2 to No. 19 (0.31 Hz to 2.96 Hz) and reduced by the fifth moment of frequency applied in the reverse direction from component No. 5 to component No. 2 (0.78 Hz to 0.31 Hz).

In Fig. 2 the normal TMFs for the 49 NTS and 42 GULF events are plotted against magnitude, using only the first term of equation 1. Separation is barely complete. In Fig. 3 the same events are shown using the MMF algorithm and the values ( $J=3$ , and so on) listed above. Good separation now exists, when the axis of projection of the two populations is taken to be the least squares line through the explosion points. Figs. 2 and 3 also indicate the inverse magnitude dependence of the TMF and MMF—for smaller events a larger proportion

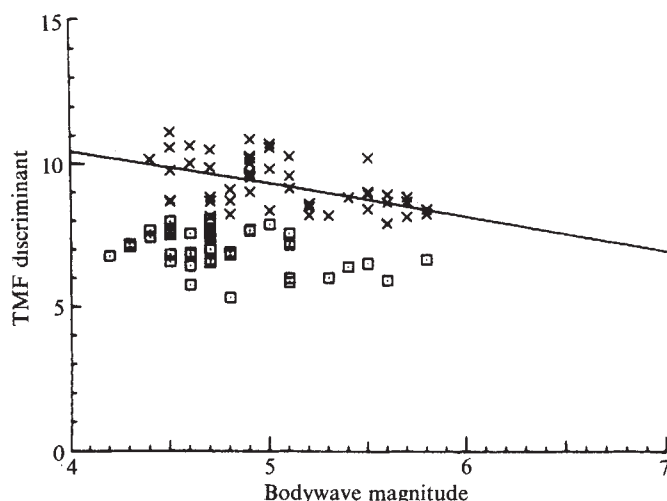


Fig. 2 Plot of normal third moment of frequency (TMF) against body-wave magnitude for the events of Fig. 1.  $\times$ , NTS;  $\square$ , GULF.

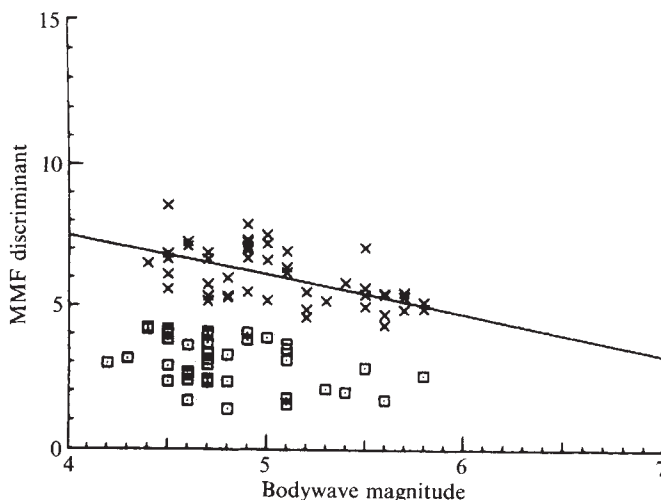


Fig. 3 Plot of the algorithm suggested here (MMF) against bodywave magnitude showing improved separation over Fig. 2.  $\times$ , NTS;  $\square$ , GULF.

of energy is concentrated at the higher frequencies than for larger events.

I believe that these two populations provide a good preliminary test of the method, because the GULF population originated entirely at Third Zone distances from Yellowknife ( $31^\circ$  to  $38^\circ$ ), whereas NTS is only  $25.3^\circ$  from Yellowknife, with the result that the explosion signals may have lost a higher proportion of their higher frequency energy in their shallower travel path, thus tending to decrease the power of the discriminants. But source regionalization is very important in SP spectral studies<sup>7</sup>. So this method must be tested for many more source areas and paths before statistical conclusions regarding its efficiency may be drawn.

When seventeen other California, Baja California and Nevada events (USA) are added to the GULF population, four of them are found to overlap the NTS events. All four are less than  $25.3^\circ$  distant from Yellowknife (two in Nevada and two in Northern California) so that, as above, normal third zone or "source window" criteria cannot be applied. Not surprisingly, none of the overlapping events is discriminated by plotting TMF or MMF against complexity.

Anglin and Israelson (personal communication) and others (Lincoln Labs) have found that multi-station application of a discriminant may increase the power of the discriminant considerably. Data for most of the events considered here have been acquired from an array at Third Zone distance from NTS; the small array that was operated by AFTAC at Mould Bay, Canada (NPNT), for several years. It will be interesting to see whether the situation regarding the overlapping earthquakes will be improved by use of Third Zone data near the NTS-Yellowknife azimuth.

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## How and Why to Record Broad Band Seismic Signals

MOST seismological observatories operate narrow band recording systems that record only that part of the seismic spectrum in which the signal/noise ratio is usually a maximum. These systems are ideal for detecting small seismic signals (say body wave magnitude  $m_b$  4.0 to 5.0) such as those generated by underground explosions of a few kton but have the disadvantage that the recorded signals are distorted versions of the true ground motion. To study seismic source functions particularly from body waves requires a broad band (BB) system such as the Kirnos system operated in the USSR<sup>1</sup> which has a constant magnification over the range 0.1 Hz to 10 Hz. The drawback of such systems is that the recording band includes the strong microseismic peak at around 0.17 Hz and thus only relatively large signals, above  $\sim m_b = 5.5$ , are recorded. Here we describe how both broad band and narrow band recordings can be obtained from a single conventional long period (LP) seismometer. The chief cost of adding a broad band system to an established observatory is then the provision of a suitable recorder. We also try to demonstrate the advantages of BB recording.

The Kirnos seismograph is an electromagnetic seismometer with a natural frequency ( $f_0$ ) of 0.04 Hz and damping factor ( $n$ ) of 0.5 loosely coupled to a heavily damped galvanometer ( $n=8.0$ ,  $f_0=0.833$  Hz). We obtain the Kirnos response by electronically shaping the output of a Geotech S-11 seismometer ( $n=0.7$ ,  $f_0=0.05$  Hz). This seismometer is manufactured with two magnet-coil assemblies fixed to the boom, the output from one of which is used for both damping and conventional LP output. The output from the second coil is fed through a high impedance integrated circuit operational amplifier with a gain of 40, then through a specially designed low pass active filter to a second amplifier before being recorded on magnetic tape. This produces a Kirnos response with an effective gain of about 1,000. In addition to the Kirnos output we use the same magnet-coil assembly to produce a short period (SP) response and so from a single seismometer obtain LP, SP and BB records. A block diagram of the system is given in Fig. 1.

Fig. 2 shows examples of P signals from a shallow earthquake, a deep earthquake and an underground explosion recorded on LP, SP and BB systems derived from the same seismometer. On the BB channel the signal from the deep earthquake suggests that the source function is a simple impulse, in contrast with the complex signals recorded on the SP channel. Similarly, the first two seconds of the explosion record suggest that the explosion source function is an impulse. This agrees with close-in observations of explosion sources, but unlike the deep earthquake, direct P is followed by the surface reflexion pP so that the signal consists of a positive impulse followed by a negative impulse (pP is almost identical to P but of opposite sign). On the SP recording the source function and P and pP are obscured by the distortion introduced by the recording system.

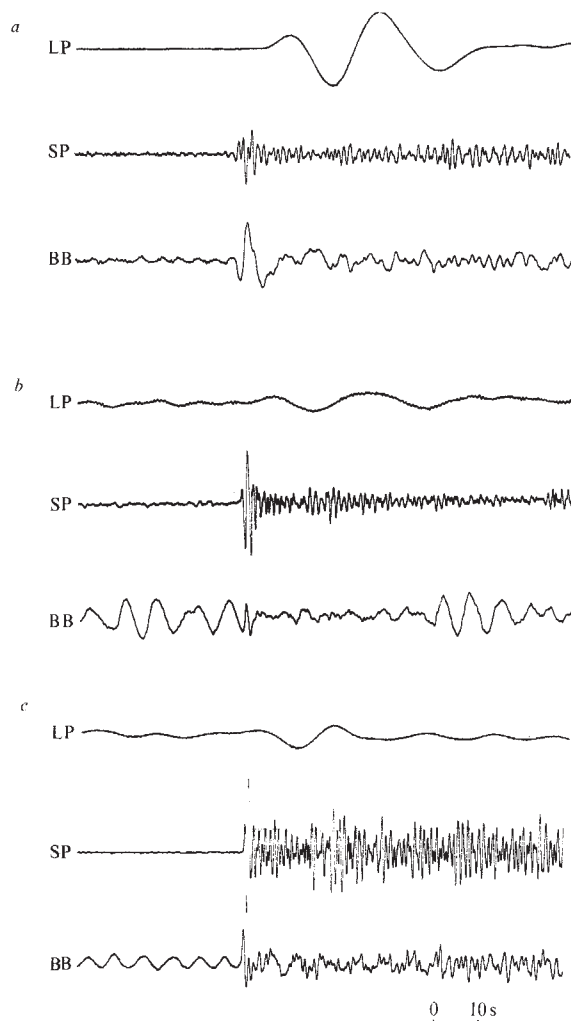


Fig. 2 P waves recorded on the three systems which are derived from the output of one long period seismometer. *a*, E. Siberia, May 18, 1971.  $h=N$ ;  $m_b$  5.8. *b*, Hindu Kush, January 20, 1972.  $h=213$ ;  $m_b$  6.0. *c*, Novaya Zemlya, October 14, 1970.  $h=0$ ;  $m_b$  6.7.

Finally, we note that for the shallow earthquake the source function is not obvious but the BB signal has a lower predominant frequency than the BB recording of the explosion. This difference in frequency content is much less obvious on the SP recordings. These spectral differences are characteristic of all explosions and shallow earthquake signals we have so far observed on the BB system so it provides a rapid method of identifying explosion signals—when the explosions are large enough to be detected.

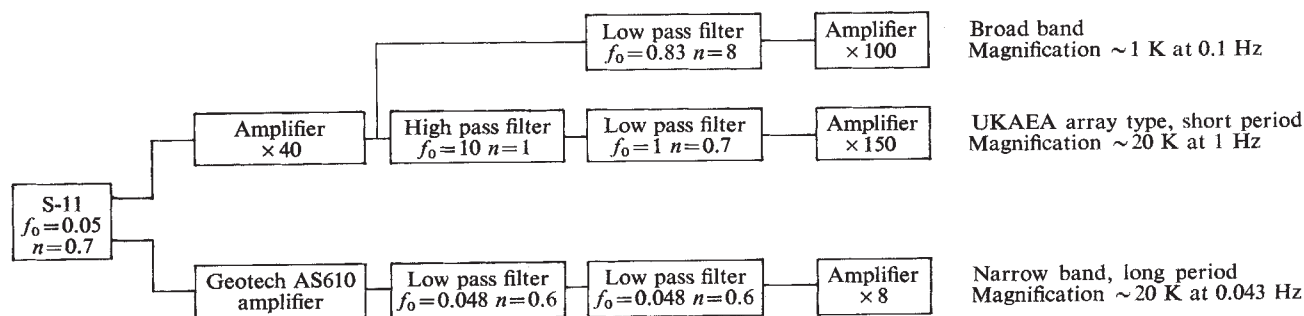


Fig. 1 Block diagram of multiple output recording system. Magnification is quoted when used with a pen recorder at a sensitivity of 1 cm/V.



Our original reason for installing a BB system was to try to reconcile disagreements on body wave magnitudes ( $m_b$ ) between seismologists in the east who measure  $m_b$  on BB instruments and those in the west who measure  $m_b$  on narrow band SP instruments. According to the SIPRI report<sup>2</sup>  $m_b$  measured on (eastern) BB instruments ( $m_E$ ) is about 0.6 magnitude units higher than  $m_w$ , the body wave magnitude measured for the same events on (western) narrow band SP systems. The first results from our study are in general agreement with the SIPRI result but we find that  $m_E - m_w$  seems to be a function of magnitude. Using  $m_E$  and  $m_w$  measurements from 62 earthquakes the relationship is

$$m_w = 0.89 m_E + 0.13$$

so that the magnitudes around  $m_b = 4.0$ ,  $m_E - m_w$  should be about 0.3 magnitude units, whereas around  $m_b = 7.0$ ,  $m_E - m_w$  is about 0.6. The reason for this increase in  $m_E - m_w$  with magnitude is probably that at low magnitudes the peak in the energy spectrum of the body waves lies around 1 Hz so that both SP and BB systems measure magnitude at a frequency near the peak in the spectrum. At high magnitudes the peak in the energy spectrum will usually be outside the pass band of the SP system but not of the BB system so that the BB recordings give a much higher  $m_b$  than the SP system, although Kondorskaya and Aponovich<sup>3</sup> suggest that the peak in the energy spectrum for body waves may be moving out of the pass band of the BB system at around  $m_E = 6.5$ .

There are two important consequences of this dependence on  $m_E - m_w$  on magnitude: first,  $m_w$  is not a reliable measure of the energy in an earthquake and, second, conclusions drawn from magnitude/frequency relationships are likely to be in error when based on  $m_w$ . The frequency of occurrence of earthquakes will appear to decrease more rapidly with increasing magnitude if magnitude  $m_w$  is used rather than  $m_E$ . Conversely, extrapolation in low magnitudes using magnitude/frequency relationships will predict too many events if  $m_w$  is used and not  $m_E$ . These differences in the magnitude/frequency relationships depending on whether  $m_w$  or  $m_E$  is used may explain some of the disagreements at the Geneva technical discussion in 1959 on banning nuclear tests (see, for example, Riznichenko<sup>4</sup>).

Because of the advantages of the BB system our group will

deploy a 4 element array to assess its value for suppressing microseismic noise.

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## Plate Tectonics and the Hercynian Orogeny

NICOLAS<sup>1</sup> has proposed that the Hercynian Orogeny in Europe was of Andean type. He assumes the presence of a Tethyan Ocean separating Southern Europe from Africa in the Palaeozoic, and in his reconstruction Africa and Europe are already affixed to North America. There is, however, no evidence for the existence of a Tethyan Ocean in the Palaeozoic but there is evidence<sup>2</sup> that Africa did not collide with North America until well into the Carboniferous. Moreover, there are good reasons to suggest contiguity of Southern Europe and Africa throughout the Palaeozoic<sup>3,4</sup>. On the other hand, there is good faunal evidence for the existence of a Mid-European Ocean separating Northern from Southern Europe in the Ordovician (ref. 5 and Burrett, in preparation) and in the Devonian<sup>6</sup>. In Silurian deposits typically Gondwanaland trilobites such as *Burmeisteria* are found to the south of this ocean and the giant *Monograptus* is found in Morocco, the Pyrenees and

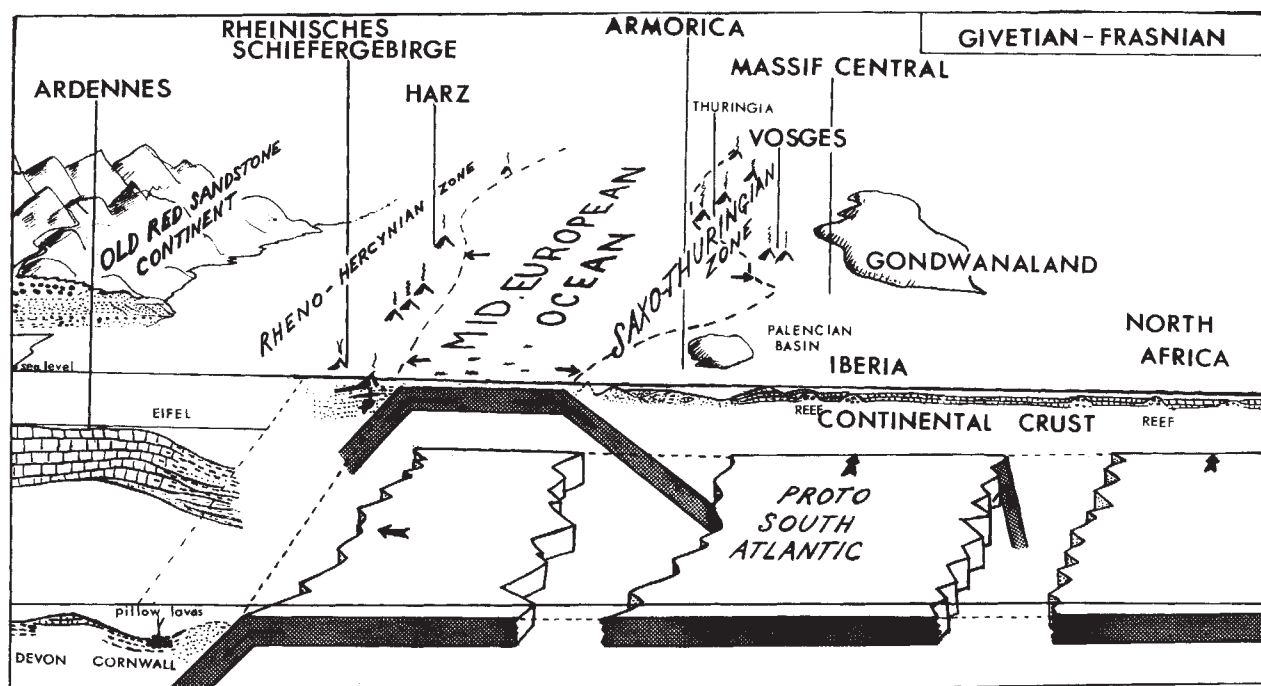


Fig. 1 Diagrammatic view of Europe during the Givetian-Frasnian. Not to scale.

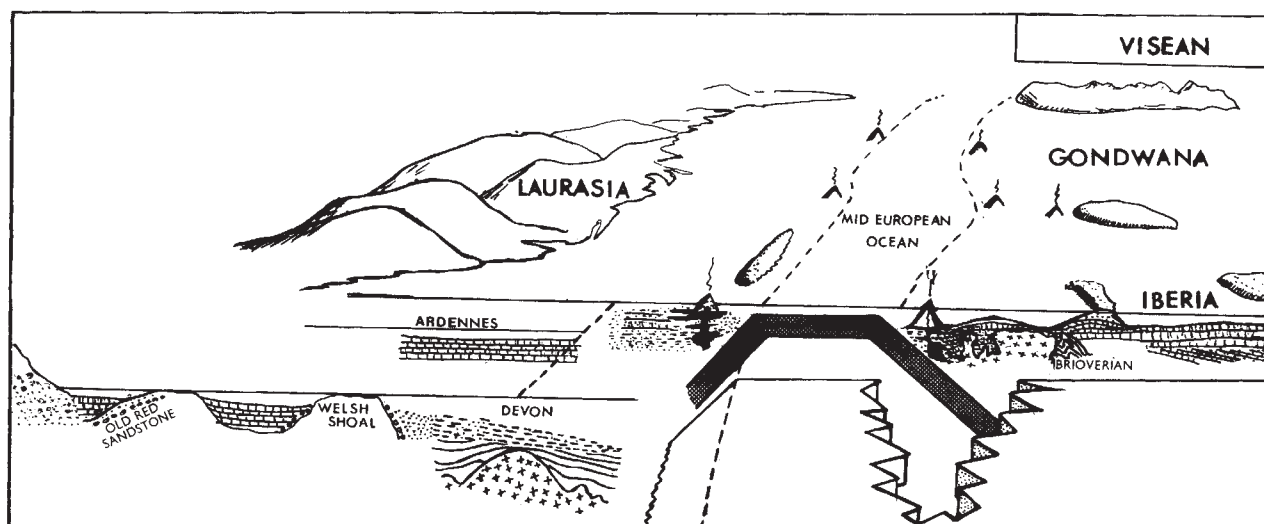
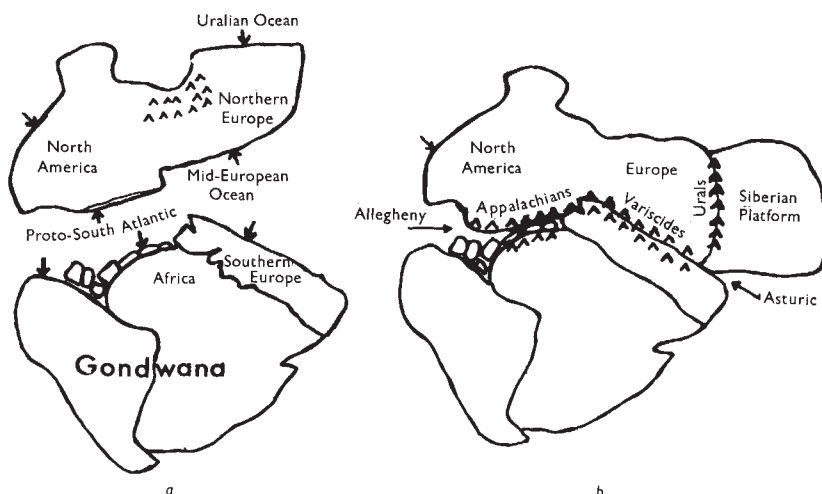


Fig. 2 Diagrammatic view of Europe during the Visean. Not to scale.

Fig. 3 Diagram showing fusion of Pangaea during the Allegheny and Asturic orogenies. *a*, Middle Devonian. *b*, Upper Carboniferous suturing.



Sardinia<sup>7</sup>. It is suggested that this Mid-European Ocean contracted during the Devonian and Carboniferous (Figs. 1 and 2) causing the collision of Northern Europe with Southern Europe and the climactic Asturic deformation in the post-Westphalian.

The line of suturing is marked by faunal differences on either side of it and by the distribution of basic volcanics, Lydian Stone and deep-water sediments<sup>8-16</sup>. In the Givetian-Frasnian of Northern Europe, deep-water sediments are found in Cornwall and Devon<sup>17</sup> associated with basic volcanics. These may be taken to indicate at least proximity to a subducting trench environment. In the Harz and Rheinisches Schiefergebirge, deep-water shales and turbidites pass laterally into spilites and diabases. This situation is also found in Southern Europe in Bohemia, Thuringia and the Vosges. Deep-water sediments without associated volcanic rocks are found in Armorica, the Massif Central and the Palencian Basin. Tuffs are, however, common. In the Lower Carboniferous volcanism is found in all of these areas, on both sides of the postulated Mid-European Ocean (Fig. 2), suggesting the presence of two subduction zones from at least the Lower Devonian into the Carboniferous. However, Southern Europe had another subducting margin along its western flank. This was the eastern edge of the Southern Proto-Atlantic Ocean. Therefore, beneath Southern Europe there were two subducted slabs of oceanic crust, which might be expected to have produced profound thermal effects. Indeed, Southern Europe displays such evidence of thermal metamorphism

especially where the two slabs were in close proximity, as in the Armorican Massif. Here there are large masses of migmatite, although the thermal metamorphism was only slightly less in the Massif Central. In Northern Europe metamorphism is weak, granitization non-existent and the Stockwerk folding resembles that of the Paratectonic zone of the Caledonides<sup>18</sup>. Generally, in Northern Europe, the Vergenz of the folds is towards the north, whereas it is to the south in Southern Europe.

The evorogenic phase can be identified as Stille's Asturic phase (within the uppermost Carboniferous) and this is coeval with the Allegheny episode in the west of the central and southern Appalachians<sup>19</sup>. Thus the post Westphalian Asturic-Allegheny folding marks the fusion of Pangaea (Fig. 3); Tethys formed in the Triassic by rifting through Southern Europe spreading into an eastward yawning sphenochasm<sup>20,21</sup>.

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## Azimuth Angle Dependence of Equatorial Ultraviolet Airglow

AN ionization chamber and an EUV solar blind photomultiplier were flown to an altitude of 103 km on a Dauphin rocket launched from Kourou, French Guiana (5° N; 35° E), on September 14, 1971, at 0020 (local time). The ionization chamber had a 15° field of view and was filled with Cs<sub>2</sub> with an MgF<sub>2</sub> window; the resulting bandpass is 1150 Å–1250 Å. The photomultiplier, field of view 5°, was enclosed in a sealed box filled with O<sub>2</sub> which transmits UV radiation only through 3 windows between 1150 Å and 1250 Å (ref. 1).

The rocket was spinning, thus allowing observations in all azimuthal directions within ±15° from the horizontal plane. The signals given by the two detectors are consistent throughout the flight. The results presented here are those provided by the photomultiplier. Our preliminary analysis reveals several

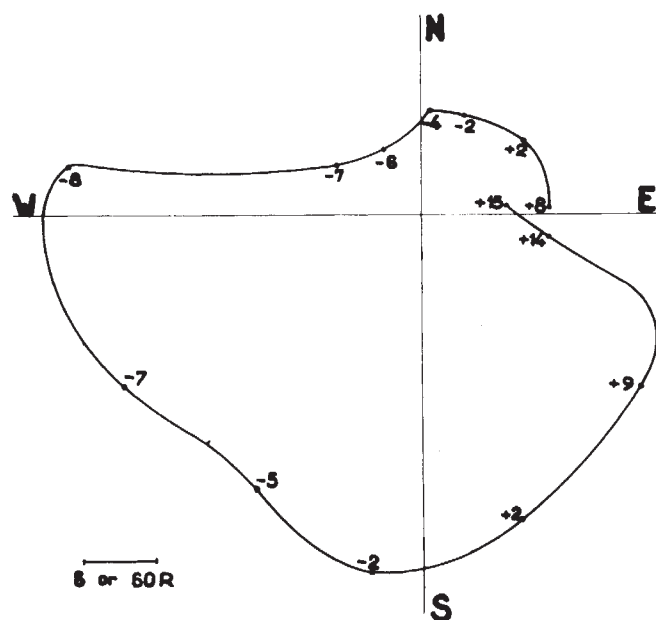


Fig. 1 Observed intensity at 102 km. Radiation intensity at a given altitude as a function of angle of observation. The positive (negative) numbers indicate the value of elevation angle above (below) the horizontal plane.

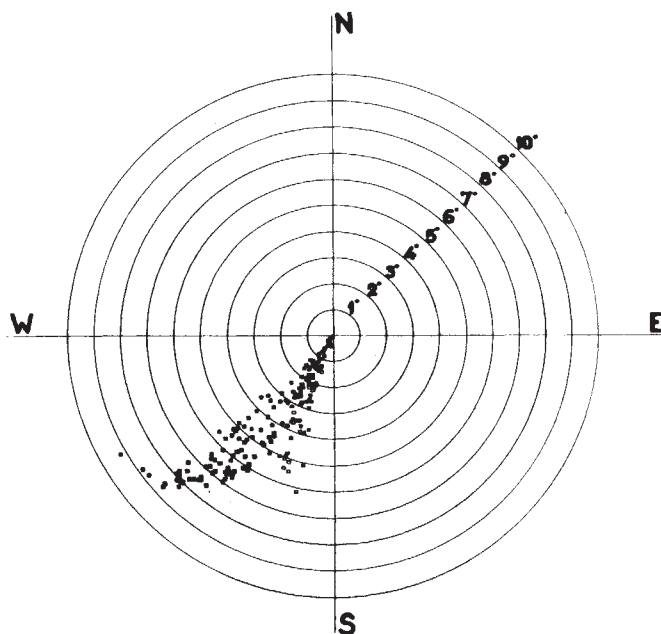


Fig. 2 Direction of maxima. Concentric circles represent absolute intensity. Black squares correspond to positive elevation, white squares for negative elevation.

interesting features: first, the observed radiation is strongly dependent on azimuth angle (Fig. 1); second, the maxima of radiation are, for all scans, located in the south-west direction (Fig. 2), and the directions of maximum are slightly different when the experiment is looking down or up; third, the radiation intensity is independent of elevation angle, suggesting that the glow is located between 75 and 105 km in altitude.

Because of uncertainty in in-flight absolute calibration, we can at present only place limits of 30 and 300 Rayleigh between which the maximum intensity must lie.

Calculations are being made to explain the origin of this unexpected emission, which is attributed to Lyman  $\alpha$  emission of atomic hydrogen atoms; these atoms must be excited by electronic collisions because multiple scattering of resonance radiation does not seem possible.

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## Radiography with 160 MeV Protons

FOR a long time, it has been the practice among nuclear physicists to take "radiographs" with beams of accelerated light nuclei to determine the location of detector targets and magnet edges relative to the position of the beam. Sharp outlines of even thin objects, for example wires of 1 mm diameter, could be clearly seen with their edges flanked by a bright line outside the edge and a dark line inside it.

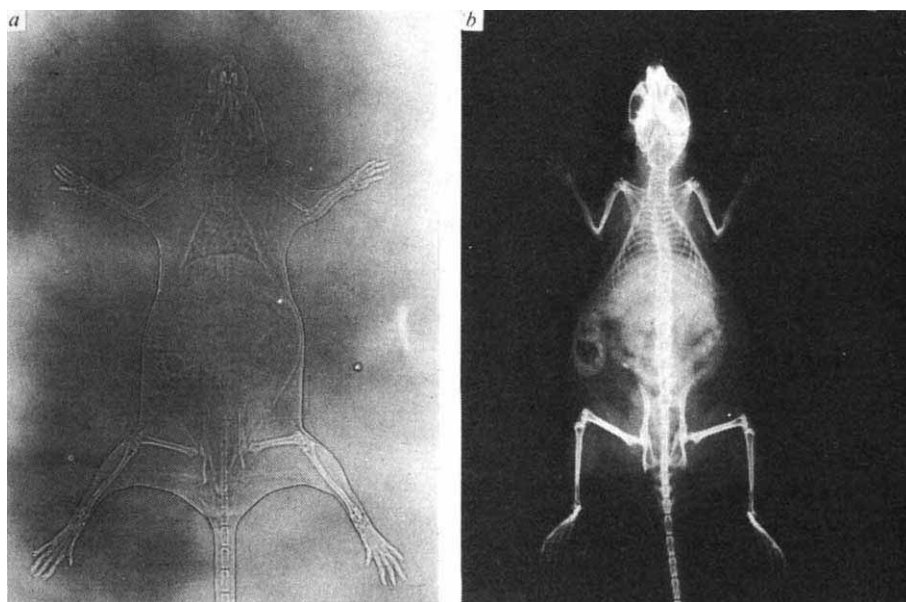


Fig. 1 *a*, Proton radiograph of a mouse with its feet 2.5 cm from the film and its back in contact with the film. *b*, X-ray radiograph of the same mouse (22 kV on tungsten). Both *a* and *b* have undergone two reversals in processing and so are of the same shading as the original radiograph.

Here we explain this phenomenon and also present some experimental proton radiographs which show that the technique is a powerful tool which complements, and in some instances even supplements, X-ray radiography. The observed dark and light bands can be explained as follows (C. Whitehead, private communication).

When a film is placed a short distance behind an edge formed by a thin layer of material and uniformly illuminated by a parallel beam of proton, particles which have passed through the layer remote from the edge produce a uniform intensity on the film since every point receives an equal number of Coulomb scattered particles from its own small area of the layer. Because most particles are scattered into a narrow forward cone, the intensity well within the shadow of the edge is the same as that well outside it ( $I_0$ ) where no scattering occurs.

At the edge, however, only half the scattered intensity is obtained because half the normal area from which scattering occurs is outside the edge. An infinitesimal distance outside the edge, the intensity is therefore  $1.5 I_0$  composed of an intensity  $I_0$  of unscattered particles which missed the layer and an intensity  $0.5 I_0$  scattered from the layer. Immediately inside the edge there is only the intensity  $0.5 I_0$  entirely composed of scattered particles. This gives an intensity discontinuity of magnitude  $I_0$  at the edge.

A striking feature which is experimentally observed is that the change of intensity is independent of the thickness of the layer. The relaxation length of the intensity discontinuity is determined by the distance of the film from the edge and by the average (root mean square) projected scattering angle  $\theta_s$ , given, for singly charged particles, by

$$\theta_s = \frac{15}{pc\beta} \sqrt{\frac{t}{x}} \quad (1)$$

where  $p$  is the particle momentum in MeV/c,  $t$  is the thickness of material comprising the edge, and  $x$  is the radiation length

for the material.  $x$  varies from 18.5 cm for C to 1.8 cm for Fe and 0.58 cm for Pb. The value of  $pc\beta$  is 300 MeV for the 160 MeV extracted proton beam of the Harwell synchro-cyclotron. Thus for iron of thickness  $5 \times 10^{-3}$  cm,  $\theta_s = 2.7 \times 10^{-3}$  rad.

Provided the projection on the film  $l/\theta_s$ , where  $l$  is the distance of the edge from the film, is greater than about  $5 \times 10^{-3}$  cm, the predicted discontinuity is clearly observed on an X-ray film but, contrary to expectation, for smaller  $l/\theta_s$  the discontinuity diminishes.

A feature of proton radiography is, apart from the change in pattern at the edge, that there is no intensity change due to thickness of the object and it is this feature which renders the scattering radiographs so different from those taken with X-rays or neutrons or by self-activity (autoradiographs).

The edge pattern of a detail seen through a layer of material suffers degradation, however, due to multiple scattering in the layer which must be folded into the edge pattern to get the resulting distribution. This effect sets a limit to the thickness of material which can be examined with a given proton energy quite apart from the total range of the particle which is, for instance, 20 to 30 g cm<sup>-2</sup> for 160 MeV protons.

Multiple scattering in a layer of thickness  $t$  in contact with a film gives a relaxation length  $t\theta_t/\sqrt{3}$  where  $\theta_t$  is the average (root mean square) scattering angle given by equation 1. A detail of thickness  $d$  on the surface gives a relaxation length  $t\theta_d$ . The criterion for negligible obscuring is at first sight  $t\theta_d > t\theta_t/\sqrt{3}$  which from equation 1 implies  $d > t/\sqrt{3}$ . This is unduly restrictive because in practice an edge of  $d = t/50$  can be seen with steel having  $t = 6$  mm. Empirically then this means that a realistic criterion of visibility is

$$\theta_d > \frac{\theta_t}{\sqrt{50}} \quad (2)$$

If we further require the detail to appear as clear as possible before suffering obscuring we can set  $t\theta_d = 5 \times 10^{-3}$  cm. Hence



we have a limiting thickness given by

$$t\theta_i < \sqrt{50} \times 5 \times 10^{-3} \text{ cm}$$

$$\text{or } t\theta_i < 35 \times 10^{-3} \text{ cm} \quad (3)$$

Table 1 shows a set of values of  $t$  deduced from equation 3 for various materials and proton energies. Below 50 MeV the range of protons limits the thickness which can be examined and above 25 GeV the nuclear interaction length (14 cm of Pb) sets the limit. Muons would not of course be subject to this high energy limitation but they are not available in sufficient intensity. Electrons are limited to thicknesses approximately equal to the radiation length and so are less useful than protons in general. For the very light nuclei the difference, however, is not very great. Table 1 also shows that medical radiography is feasible with the 7 GeV proton synchrotron, Nimrod.

**Table 1** "Maximum" Thickness (cm) of Material which can be Examined with Good Definition by Proton Scattering Radiography as a Function of Proton Energy

| Proton energy (MeV) | Polythene $\rho = 0.92$ (g/cm <sup>3</sup> ) | C $\rho = 1.6$ Graphite<br>Radiation length (cm) |      |     |     |     |     |
|---------------------|----------------------------------------------|--------------------------------------------------|------|-----|-----|-----|-----|
|                     |                                              | 47.9                                             | 26.5 | 8.9 | 1.8 | 1.2 | 0.6 |
| 50                  | 97.5                                         | 1.4                                              | 1.1  | 0.8 | 0.5 | 0.4 | 0.3 |
| 160                 | 296.7                                        | 2.8                                              | 2.3  | 1.6 | 1.0 | 0.8 | 0.7 |
| 600                 | 966.0                                        | 6.2                                              | 5.1  | 3.6 | 2.1 | 1.8 | 1.4 |
| 7,000               | 7,830                                        | 25                                               | 21   | 14  | 8   | 7   | 6   |
| 25,000              | 25,900                                       | 56                                               | 46   | 32  | 19  | 16  | 13  |

Fig. 1 shows a proton scattering radiograph of a mouse taken in this manner and also, for comparison, X-radiograph of the same mouse. The proton radiograph shows the outline of the skin and internal membranes of the animal at the same time as the bone, which X-radiographs can only do if special efforts are taken. Induced activity is not a serious problem at 160 MeV but the dose to living tissue, using standard medical X-ray film (Kodirex is some 50 times more sensitive than that used here), is close to the maximum permissible level.

We hope that this type of radiography may complement other forms for applications which make use of the special attributes which these radiographs possess.

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same enzyme system that acetylates isoniazid and sulphamethazine. Unlike acetylisoniazid and acetylsulphamethazine, however, MADDS is rapidly deacetylated in man. Consequently, constant plasma ratios of acetylated to parent drug are found almost immediately after dosage with DDS, and significantly higher ratios are found in rapid acetylators of isoniazid or sulphamethazine than in slow acetylators. The half-life of DDS in the body, unlike that of isoniazid, seems to be unrelated to the speed at which it is acetylated.

In the chemotherapy of tuberculosis the efficacy of isoniazid can be influenced by the acetylator phenotype of the patient when suboptimal regimens are used, particularly when isoniazid is given intermittently<sup>2,3</sup>. To determine whether the rate of acetylation of DDS could be of prognostic significance in the treatment of leprosy, retrospective assessments were made of the acetylation status of two groups of patients most likely to have received suboptimal dosage of DDS. One group consisted of six patients who had participated in a pilot clinical trial of 1 mg DDS daily<sup>4</sup> and who responded initially as well as patients receiving the conventional dose of 100 mg DDS daily. The other group consisted of twenty-three patients who had relapsed after six or more years of treatment with conventional doses of DDS owing to the appearance of DDS-resistant strains of *Mycobacterium leprae*<sup>5-7</sup>.

In this study the acetylator phenotype of each patient was judged on the capacity to acetylate isoniazid. Plasma concentrations of isoniazid were measured fluorimetrically<sup>8</sup> 6 h after an oral dose of 40 mg/kg<sup>0.7</sup> of the drug. Patients with concentrations greater than 3.75 µg/ml. were classified as slow inactivators<sup>9</sup>, and those with less than this value as rapid inactivators<sup>9</sup>. The acetylator status was confirmed by measuring the ratio of the excretion of acetylisoniazid to that of isoniazid during the first 2 h after dosage with isoniazid<sup>10</sup>.

In the six patients (three Chinese, two Malaysian and one Indian) investigated who were being treated with 1 mg DDS daily, two were rapid acetylators (one Chinese and one Malaysian). Peak DDS serum concentrations achieved with this dose of DDS were only two to eight times the minimal inhibitory concentration of the drug against *M. leprae* determined in the mouse foot-pad system<sup>11</sup>. The two rapid acetylators, however, responded to treatment during the first 4.5 months as satisfactorily as the slow acetylators, response being judged by the increase of degenerate leprosy bacilli in their skin<sup>4</sup>.

In the twenty-three patients who had relapsed with DDS resistant strains of *M. leprae*, six were slow and seventeen were rapid acetylators. DDS resistant bacteria are able to multiply in the foot pads of mice treated orally with 0.01% DDS, giving serum DDS levels equivalent to those in man receiving 100 mg DDS daily<sup>11</sup>. Twenty-one patients were Chinese and, of these, fifteen (71%) were rapid acetylators. This proportion is similar to that of 78% found among Chinese living in Formosa<sup>12</sup>.

Our results suggest that the rate of acetylation of DDS is likely to be without prognostic effect in the treatment of leprosy. Whether or not MADDS also has antileprosy activity *per se* cannot be established at present because in both man and the mouse, in which its therapeutic efficacy might be investigated, it is rapidly deacetylated to DDS<sup>1,13</sup>.

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## BIOLOGICAL SCIENCES

### Dapsone Acetylation and the Treatment of Leprosy

DAPSONE (4,4'-diaminodiphenyl sulphone, DDS) continues to be the standard drug for the treatment of leprosy. Recent studies<sup>1</sup> have shown that DDS is converted in man to mono-acetyldapsone (MADDS) in a polymorphic fashion by the

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## Natural Selection and Genetic Drift in Protein Polymorphism

THE Kimura theory<sup>1</sup> that protein polymorphism is mainly due to random genetic drift acting on a number of neutral isoalleles has been recently discussed by many authors<sup>2-4</sup>. The electrophoretic study of populations of the same species having different geographical origin and showing great fluctuations in number offers possibilities for testing the relative importance of natural selection and genetic drift in the context. We report a study of two species of mosquitoes, *Aedes aegypti* and *Aedes mariae*, for the phosphoglucosylase (PGM) locus. This is an autosomal locus belonging in *A. aegypti* to the linkage group 2 and showing in both species a very high number of electrophoretically detectable alleles (Bullini *et al.*, refs. 5 and 6, and unpublished data).

*A. aegypti*, the yellow fever mosquito, is a colonizing species ubiquitous in the tropics and subtropics and widely distributed in various temperate zones. Its successful spreading all over the world from the ancestral African forest range appears to be

**Table 2** Distribution of PGM Alleles in Population Samples of *Aedes mariae* of Different Geographic Origin

| Geographic origin        | Frequency of PGM alleles |                          |                          |                         |                         |
|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|
|                          | <i>Pgm</i> <sup>A3</sup> | <i>Pgm</i> <sup>A2</sup> | <i>Pgm</i> <sup>A1</sup> | <i>Pgm</i> <sup>B</sup> | <i>Pgm</i> <sup>C</sup> |
| Tellaro (La Spezia)      | —                        | —                        | —                        | 0.990                   | 0.010                   |
| Quercianella (Livorno)   | —                        | —                        | —                        | 0.941                   | 0.059                   |
| La Cannella (Argentario) | —                        | —                        | 0.012                    | 0.930                   | 0.058                   |
| Circeo (Latina)          | —                        | —                        | —                        | 1.000                   | —                       |
| Sperlonga (Latina)       | —                        | —                        | —                        | 1.000                   | —                       |
| Torre Capovento (Latina) | —                        | —                        | —                        | 1.000                   | —                       |
| Scauri (Latina)          | —                        | —                        | —                        | 1.000                   | —                       |
| Furore (Salerno)         | —                        | —                        | —                        | 0.983                   | 0.017                   |
| Capri Island             | —                        | —                        | —                        | 1.000                   | —                       |
| Catania (Sicily)         | —                        | —                        | 0.011                    | 0.989                   | —                       |
| Levanzo (Egadi Islands)  | —                        | —                        | —                        | 0.806                   | 0.194                   |
| Santa Lucia (Sardinia)   | —                        | 0.040                    | —                        | 0.731                   | 0.230                   |
| Bosa (Sardinia)          | —                        | 0.125                    | —                        | 0.741                   | 0.133                   |
| Cap Bon (Tunisia)        | 0.149                    | —                        | —                        | 0.851                   | —                       |
| Bizerte (Tunisia)        | —                        | —                        | —                        | 1.000                   | —                       |

strictly related to its adaptation to the human environment. On the other hand, since 1946 it has been under a very strong insecticide pressure. The distribution of PGM alleles was compared in 19 population samples represented, with a few exceptions, by material tested after one to three laboratory generations. The data are summarized in Table 1. In spite of the different geographical origin of this material from Africa, Asia, America and the Pacific region, the allele *Pgm*<sup>A1</sup>, having intermediate electrophoretic mobility, was always present in high frequency, being the most frequent in 17 of the 19 populations tested. Moreover, this allele is the only one present in the three non-polymorphic samples from Puerto Rico, Dacca and Karachi respectively.

*A. mariae* is the western Mediterranean member of the *mariae* complex (Coluzzi and Bullini<sup>7</sup>). The larvae of this mosquito breed in rock pools along the coasts and are fre-

**Table 1** Distribution of PGM Alleles in Population Samples of *Aedes aegypti* of Different Geographic Origin

| Geographic origin        | Frequency of PGM alleles |                          |                            |                          |                          |                            |                          |
|--------------------------|--------------------------|--------------------------|----------------------------|--------------------------|--------------------------|----------------------------|--------------------------|
|                          | <i>Pgm</i> <sup>A3</sup> | <i>Pgm</i> <sup>A2</sup> | <i>Pgm</i> <sup>A1,2</sup> | <i>Pgm</i> <sup>A1</sup> | <i>Pgm</i> <sup>B2</sup> | <i>Pgm</i> <sup>B1,2</sup> | <i>Pgm</i> <sup>B1</sup> |
| Sierra Leone             | —                        | —                        | —                          | 0.564                    | —                        | —                          | 0.436                    |
| Ivory Coast              | —                        | —                        | —                          | 0.122                    | 0.878                    | —                          | —                        |
| Niangoloko (Upper Volta) | —                        | —                        | —                          | 0.234                    | 0.766                    | —                          | —                        |
| Garki (Nigeria)          | —                        | 0.006                    | 0.023                      | 0.599                    | 0.276                    | —                          | 0.099                    |
| Bongoyo (Tanzania)       | 0.033                    | 0.048                    | —                          | 0.881                    | —                        | 0.026                      | 0.011                    |
| Dar es Salaam (Tanzania) | —                        | —                        | —                          | 0.844                    | —                        | 0.156                      | —                        |
| Dodoma (Tanzania)        | —                        | 0.441                    | —                          | 0.443                    | 0.116                    | —                          | —                        |
| Mwanza (Tanzania)        | —                        | —                        | —                          | 0.759                    | —                        | —                          | 0.241                    |
| Nambunga (Tanzania)      | —                        | 0.304                    | —                          | 0.609                    | 0.087                    | —                          | —                        |
| Newala (Tanzania)        | —                        | 0.117                    | —                          | 0.883                    | —                        | —                          | —                        |
| Oyster Bay (Tanzania)    | —                        | 0.144                    | —                          | 0.670                    | 0.186                    | —                          | —                        |
| Uvinza (Tanzania)        | —                        | 0.213                    | —                          | 0.441                    | 0.346                    | —                          | —                        |
| Comore Islands           | —                        | —                        | —                          | 0.958                    | 0.042                    | —                          | —                        |
| Dacca (Bangladesh)       | —                        | —                        | —                          | 1.000                    | —                        | —                          | —                        |
| Karachi (Pakistan)       | —                        | —                        | —                          | 1.000                    | —                        | —                          | —                        |
| Kuala Lumpur (Malaysia)  | —                        | 0.149                    | —                          | 0.829                    | —                        | —                          | 0.022                    |
| Bangkok (Thailandia)     | 0.002                    | 0.014                    | —                          | 0.750                    | 0.016                    | —                          | 0.181                    |
| Tahiti                   | —                        | 0.072                    | —                          | 0.752                    | —                        | —                          | 0.176                    |
| Puerto Rico              | —                        | —                        | —                          | 1.000                    | —                        | —                          | —                        |



quently swept away by sea storms. The distribution of the *Pgm* alleles was studied in 15 geographically isolated populations representative of almost the whole range of the species and collected directly from the field. The data, given in Table 2, show that the *Pgm<sup>B</sup>* allele is always the most frequent (or the only one present) in all the populations tested. In contrast to this, different *Pgm* alleles predominate in the other two siblings of the complex: *Pgm<sup>A1</sup>* in *A. zammitii* and *Pgm<sup>C</sup>* in *A. phoeniciae* (Coluzzi, Bullini and Bianchi Bullini, refs. 8 and 9, and unpublished data).

The findings are in good agreement with the observations of Prakash *et al.*<sup>3</sup> in *Drosophila pseudobscura*, suggesting that protein polymorphism is not primarily influenced by random genetic drift acting on a number of neutral isoalleles as suggested by Kimura, but is most probably caused by some form of balancing selection.

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## Evidence for a Primary Association between Immunoblasts and Small Gut

SINCE the observations of Gowans and Knight<sup>1</sup> it has been shown that many of the lymphoid blast cells (immunoblasts) that appear in the thoracic duct lymph of rats after immunization of the caudal lymph nodes with a variety of antigens "home" to the lamina propria of the small gut<sup>2</sup>, where they differentiate into plasma cells<sup>3</sup>. The immunological specificity of the immunoblasts, however, does not seem to affect their affinity for the small gut<sup>2,3</sup>. Recent work<sup>4</sup> has shown that immunoblasts home just as readily to the small gut of unsuckled, neonatal rats obtained by Caesarian section, in which the gut is sterile and presumably antigen-free, and where there is no influence of endogenous or maternal colostral immunoglobulins. The entry of immunoblasts into the lamina propria must involve the transmigration of these cells across the vascular endothelium. The first event in such a process may be the engagement of a receptor on the surface of the immunoblast with a complementary group on its endothelial surface. We have studied the entry of immunoblasts into "free" grafts of syngeneic foetal gut, implanted subcutaneously, and the entry of xenogeneic immunoblasts into normally situated gut.

Pure-line, barrier-maintained, male hooded rats from our colony were immunized in the hindquarter regions with multiple subcutaneous (SC) injections of killed *Corynebacterium parvum* so that on cannulation of their thoracic ducts 3–4 days later 10–15% of the cells in the lymph were immunoblasts. The large lymphoid immunoblasts were labelled by incubating the washed lymph cells with radioactive precursors of DNA (either <sup>125</sup>I-iodo-deoxyuridine (IUDR) or <sup>3</sup>H-thymidine) and were injected intravenously (IV) into recipient rats (in doses of 1–5 × 10<sup>7</sup> total cells contained in 1.0 ml. BSS) and their distribution in the tissues determined by scintillation counting

and autoradiography. The details of these procedures have been published<sup>2–4</sup>.

Fourteen adult male rats received subcutaneous implants of 3 cm lengths of syngeneic, foetal (18 day) small gut over the abdomen according to the method of Zinzar *et al.*<sup>5</sup>. Five of the fourteen rats also received an implant of a 2 cm length of foetal large gut. After 2–3 weeks the rats received an IV injection of immunoblasts labelled with <sup>125</sup>IUDR. The rats were killed 24 h later and the amount of radioactivity in the implants was compared with that of an equal length of the normal adult gut from the same animal.

As in previous studies<sup>2,3,6</sup>, about 30% of the injected immunoblasts entered the lamina propria of the normal small gut and less than 5% entered the normal large gut. The specific radioactivity of the fourteen implants of foetal small gut (corresponding to the number of immunoblasts per unit length) averaged 75% (standard error=6.5) of that of the normal, adult, *in situ* gut. The ratio of the specific activities of the implants of foetal small and large gut was the same as that of the normal adult small and large gut. Histology showed that the structure of the implanted gut was perfectly preserved and well vascularized. Autoradiographs prepared from the tissues of a further two rats that had received immunoblasts labelled with <sup>3</sup>H-thymidine confirmed that the radioactivity was localized in immunoblasts which had extravasated into the lamina propria of both the normal and implanted gut. Although the implants of foetal small gut received their blood supply from the musculo-cutaneous tissues of the abdominal wall and were thus isolated from the vascular, neural and environmental influences available to normally situated gut, they nevertheless received a similar proportion of the available immunoblasts. It was concluded that the vascular endothelium associated with the small gut possesses the ability to transmit immunoblasts as an intrinsic property which is not dependent on the local environment or gross anatomical integrity.

To determine whether a species difference between the immunoblasts and the gut would affect their extravasation, rat immunoblasts were injected into mice. Five adult, male C57/BL mice were killed 24 h after they had received an IV injection of rat immunoblasts labelled with <sup>125</sup>IUDR, and their major organs were assayed for radioactivity. Again the small gut contained the largest amount of activity (12–15% of the injected dose). Autoradiographs of the small gut of a further two mice which had received cells labelled with <sup>3</sup>H-thymidine confirmed that the radioactivity was localized in immunoblasts which had extravasated into the lamina propria.

Sheep immunoblasts were obtained by cannulating the efferent duct of the prefrontal node after antigenic stimulation with *C. parvum*<sup>5</sup>, and were labelled with <sup>125</sup>IUDR. When these labelled sheep cells were injected IV into adult rats they were rapidly sequestered in the liver and spleen and destroyed; much of the <sup>125</sup>I was excreted in the urine, and after 24 h no intact labelled cells could be found in any of the rat tissues. It was concluded that the immune system of the adult rat could, perhaps with the aid of naturally occurring opsonic antibody, recognize and react immediately against the cells from an unrelated species. The experiment was repeated using as recipients newborn rats which are agammaglobulinaemic, and in which the RES is much less able to sequester foreign material<sup>7</sup>. Six newborn rats from the same litter were killed 24 h after they had received IV injections of sheep immunoblasts labelled with <sup>125</sup>IUDR. This time very little of the label was excreted in the urine and, although 30–40% of the activity was located in the liver and spleen combined, 10–15% of the injected dose was recovered from the small gut. Autoradiographs showed that intact, labelled immunoblasts had extravasated into the lamina propria.

Although the efficiency of entry of xenogeneic immunoblasts into the lamina propria of the small gut was somewhat less than that of syngeneic cells, the phenomenon appears to be qualitatively similar. If the putative complementary receptors on the immunoblasts and endothelial cells do exist, it would

seem that they are common to several species, and such a lack of diversity might suggest that the receptors are carbohydrates rather than proteins. This would be consistent with the evidence of Gesner *et al.*<sup>8</sup> for the nature of the surface structures of small lymphocytes which permit these cells to extravasate preferentially into lymph nodes. But whatever its molecular basis, the affinity between the immunoblasts and the small gut in xenogeneic combinations suggests a relationship established at a relatively early stage of evolution. Indeed, evolutionary considerations have led Fitchtelius *et al.*<sup>9</sup> to suggest that the mammalian gut may be a primary lymphoid organ, equivalent to the avian bursa.

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## Increased Shear Resistance of Human Erythrocytes in the Presence of Glucose

HUMAN erythrocytes are damaged when pumped through extracorporeal cardiopulmonary bypass circuits<sup>1</sup>, haemodialysis units or prosthetic cardiac valves<sup>2</sup>. Although relatively short periods of exposure can be associated with acceptably low levels of immediate erythrocyte destruction, more extended periods of recirculation may approach or exceed the regenerative capacity of the individual. In addition, the intact cells which survive the shearing process usually exhibit "sublethal" or delayed damage as manifested as a post-perfusion anaemia<sup>3,4</sup>, a decreased erythrocyte lifespan *in vivo*<sup>2</sup> and an increased rate of autolysis during incubation at 37° C *in vitro*<sup>5</sup>. These factors limit the clinical applications of mechanically assisted blood flow. This damage could be reduced if the mechanical resistance of the erythrocyte membrane to disruption or damage by hydrodynamic shear stress were increased.

The mechanical properties of fresh human erythrocytes at 25° C were investigated with a Ferranti-Shirley cone and plate viscometer having a water-cooled plate and a torsion head which measured the applied shear stress. This device subjected 0.6 ml. of a dilute erythrocyte suspension (approximately 1.5% haematocrit in isotonic or hypotonic saline) to a uniform velocity gradient of up to  $1.75 \times 10^4 \text{ s}^{-1}$ . As a non-penetrating thickener 10 to 13% w/v of dextran 500 (Pharmacia) was added to increase the viscosity of the suspension so that hydro-

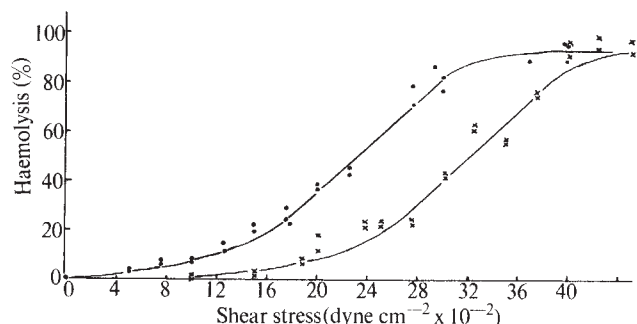


Fig. 1 Haemolysis profiles of human erythrocytes after 5 min shear at various hydrodynamic shear stresses in hypotonic (0.54%) dextran/saline containing 0 mM (●) and 30 mM (×) D-glucose.

dynamic shear stresses of up to 5,000 dynes  $\text{cm}^{-2}$  could be applied at these relatively low velocity gradients. Four drops of adult human blood were removed by finger puncture and rapidly diluted with 10 ml. of dextran/saline solution so that no additional anticoagulant was required. After shearing at the chosen stress for 5 min the sample was removed with a hypodermic syringe and 0.2 ml. added to 2.5 ml. of isotonic saline and centrifuged for 5 min at 3,000 r.p.m. in a bench centrifuge to sediment the intact cells. The degree of haemolysis was estimated from the absorbance of the supernatant at 540 nm.

Erythrocytes in isotonic media fragment, with little loss of haemoglobin, to give a characteristic distribution of haemoglobin-filled microspheres when subjected to large hydrodynamic shear stresses<sup>6</sup>. Some microspheres had diameters less than 0.5  $\mu\text{m}$  and did not sediment during centrifugation. The light scattered by these microspheres contributed a large source of error and irreproducibility to estimation of the degree of haemolysis. Consequently, the present experimental series was performed with erythrocytes in a hypotonic medium (13% dextran 500 in 0.54% saline or saline/glucose of equivalent osmotic strength) so that the individual cells were swollen but not spherised. This procedure reduced the number of microspheres formed and gave a reproducible and more meaningful estimate of the hydrodynamic shear stress ( $\tau$ ) required to disrupt mechanically the erythrocyte membrane (Fig. 1).

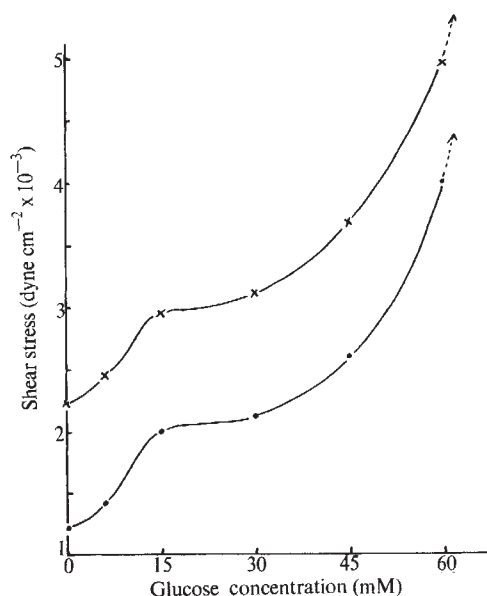


Fig. 2 A plot of the shear stress required to disrupt the weakest (●) and the average (×) erythrocytes in hypotonic (0.54%) dextran/saline as a function of the concentration of glucose in the suspending medium.



Haemolysis/shear stress profiles similar to those in Fig. 1 were obtained at each different glucose concentration. In each the experimental points could be fitted to the same curve, which differed only in its position on the shear stress axis. Since the shape of this curve shows the distribution of mechanical strengths among the heterogeneous erythrocyte population, it follows that a given concentration of glucose increases the mechanical strength of every cell, irrespective of its age, to the same extent.

Fig. 2 shows the average ( $\tau_{50\%}$ ) and "critical" ( $\tau_{crit}$ )/shear stress values obtained from each haemolysis/stress profile as a function of the glucose concentration. It can be seen that the shear stress required to mechanically disrupt human erythrocytes increases with increasing concentration of glucose. It is tempting to speculate that the two-stage nature of the "glucose protection effect" might represent two different protection mechanisms.

The clinical implications of these observations are that it might now be possible to increase the mechanical strength of the erythrocyte membrane *in vivo* and so reduce the immediate and/or sublethal damage resulting from *in vivo* or *in vitro* trauma. This approach would seem to be doubly attractive because high blood glucose levels are readily produced by infusion of glucose or the pre-operative administration of a drug similar to glucagon, and because high blood glucose levels are well tolerated and do not seem to endanger the health of the patient in the short term.

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## Role of Turgor in Plant Cell Growth

BURSTRÖM<sup>1</sup> recently criticized the generally held view that plant cell enlargement involves physical yielding of the cell wall driven by turgor forces, saying, "turgor does not cause expansion and is not the driving force . . . The driving force of any expansion is a difference in water potentials (because) expansion is due to water uptake . . . The literature on plant cell growth would certainly improve if the notion of turgor expanding the cell was abandoned and replaced by accepted equations for water balance of fluxes".

Burström fails to come to grips with the principle that irreversible increase in plant cell volume involves simultaneous water uptake (driven by a water potential difference) and cell wall yielding that depends on turgor stress, and in this sense is "driven by" turgor pressure. Lockhart<sup>2</sup> derived the basic rate equation for plant cell enlargement on the basis that during steady volume increase the rates of water uptake and cell wall yielding (expressed as a rate of volume increase) must be equal.

Recapitulating, briefly, Lockhart's principle<sup>2</sup>, let  $\dot{v}$  stand for relative rate of change in cell volume ( $dV/Vdt$ ), and let the rate of water uptake be governed by a conductivity  $L$  such that

$$\dot{v} = L \cdot \Delta\psi = L (\Delta\pi - P) \quad (1)$$

where  $\Delta\psi$  is the difference in water potential between medium

and cell,  $\Delta\pi$  the difference in osmotic potentials (osmotic pressures) of cell and medium, and  $P$  the turgor pressure.

The rate of yield of the cell wall as a function of turgor stress (expressed in terms of relative rate of change of volume of the cell wall chamber) will be denoted  $y(P)$ . For carefully studied examples, *Nitella*<sup>3</sup> and oat coleoptile<sup>4</sup> cells,  $y(P)$  has approximately the form  $\phi(P - Y)$  where  $Y$  is a yield threshold and  $\phi$  is a yielding compliance, often called "extensibility", that is,

$$\dot{v} = y(P) \approx \phi(P - Y) \quad (2)$$

Equations (1) and (2) must be equal, and when set equal and solved for  $P$  one obtains the value of turgor pressure that must prevail, during steady growth, to satisfy the requirements for simultaneous volume increase by water uptake and yield of the cell wall. For example, using the expression at the right in equation (2) we get

$$P = \frac{L\Delta\pi + \phi Y}{L + \phi} \quad (3)$$

Substituting this into either equation (1) or (2) leads to the rate equation for cell enlargement

$$\dot{v} = \phi L \cdot \frac{\Delta\pi - Y}{L + \phi} \quad (4)$$

This shows that both water permeability ( $L$ ) and wall extensibility ( $\phi$ ) affect the growth rate.

It is evident from equation (4) that, as Lockhart<sup>2</sup> pointed out, if one of these parameters is much larger than the other, then the rate becomes governed or "limited" by the smaller one; for example, if  $L$  is large compared with  $\phi$ , equation (4) reduces to

$$\dot{v} = \phi(\Delta\pi - Y) \quad (5)$$

which is the limiting case of "extensibility-controlled" expansion rate. Burström<sup>1</sup> in effect assumes this case, since he says that for plant cells "the water permeability is usually so high that these two [ $\psi$  of cell and  $\psi$  of medium] are always equal". This is a statement that  $\Delta\psi$  in equation (1) is negligible and the consequence of this is equation (5) in which wall extension and not water flux enters the practical rate equation for cell growth, contrary to Burström's recommendation.

The assumption that  $L$  is very large compared with  $\phi$  (therefore the  $\Delta\psi$  is negligible during growth) has been widely accepted, even though it is based on erroneous<sup>5</sup> interpretation, as pointed out previously<sup>6</sup>. However, the assumption is clearly valid for *Nitella*, whose  $L$  is extremely high and  $\dot{v}$  relatively slow; in this case  $P$  has been measured directly and found to equal  $\Delta\pi$ <sup>7</sup>. The assumption is also probably valid for slowly growing higher plant tissues such as auxin-stimulated potato tissue, but careful measurements of  $L$  for oat coleoptile tissue showed that during auxin-induced rapid elongation  $\Delta\psi$  was by no means negligible<sup>6</sup>. Similar results have been obtained in unpublished experiments on auxin-treated pea stem tissue by P. M. R. and it may be expected that, for these objects and perhaps for rapidly growing plant tissues more generally, equation (5) is not valid and a more general expression of the type of equation (4) must be used.

Criticizing the application by Green *et al.*<sup>3</sup> of equation (5) to growth of *Nitella* cells, Burström<sup>1</sup> failed to appreciate that, while an expression of the type of equation (2) describes the biophysics of the immediate process of wall yield, overall growth in the long term may depend on  $P$  in a different manner because of regulatory adjustments in the function  $y(P)$  that the cell can make. For example, with *Nitella* it was demonstrated that, following a sudden osmotically imposed change in  $P$  (and in resultant rate of expansion, by equation (5)),  $Y$  shifts to a new value, over one to several hours, such that the long term growth rate is much less sensitive to variation in  $P$  than is the rate of cell expansion during the period immediately after the turgor shift.

The equality between rate of volume increase and of

irreversible yielding on which equations (4) and (5) are based applies to both short- and long-term growth measurements, provided the time scale lies outside that over which changes in  $P$  (and also retarded elastic strain processes) are occurring in response to experimental perturbations. It must be kept in mind, therefore, that the values of the parameters in rate equations such as equation (4) or (5) can depend on the time scale of measurement. Contrary to Burström's objection, this constitutes no difficulty of principle and amounts simply to taking account of the physical and biological realities of the plant cell growth process.

A conceptual difficulty with equation (4) and its derivation is that, as stated, it pertains to a steady state of volume uptake and wall yielding, and does not attempt to describe the process by which  $P$  departs initially from the osmotic equilibrium value ( $\Delta\pi$ ), generating a  $\Delta\psi$  to drive water uptake, nor how  $P$  is maintained, over time, at the steady state value. This conceptual difficulty we feel probably lies at the root of Burström's<sup>1</sup> criticisms of the concept of turgor as a driving force for cell enlargement. He states that "growth . . . begins with a loosening of the wall . . ." but does not explain clearly how this generates the  $\Delta\pi$  necessary to satisfy equation (1).

For any uptake of water to occur and extend the cell wall, the cell's water potential must first fall below that of the medium. In default of changes in the cell's osmolarity the only way this can occur is if turgor pressure  $P$  falls. Without a change in volume of the cell contents (which cannot occur until a  $\Delta\psi$  exists) the only way turgor can fall is if stress relaxation (decrease of stress at constant strain,  $\Delta\sigma$ ) takes place in the cell wall. This could be caused either by some action of the cell on its wall or by some response of the cell wall to stress borne by it. Because wall stress and turgor stress constitute action and reaction,  $\Delta\sigma$  will be accompanied by a turgor relaxation  $\Delta P$  and a corresponding reduction of cell water potential. Because of the resulting  $\Delta\psi$  between cell and environment, the cell will begin to absorb water osmotically, and its volume will increase. The consequent extension of the cell wall will be governed in part by continuation of the same process that produced the initial stress relaxation. In the ensuing steady state of constant turgor stress and constant rate of irreversible strain the process that initially produced stress relaxation will govern the parameters of the extension rate (equation (2)).

Clearly stress relaxation is the primary event in cell enlargement, whereas water uptake, volume increase and extension (strain) of the cell wall are secondary. Since the rate of stress relaxation increases with stress itself the existence of turgor stress is a prerequisite for cell enlargement, and turgor pressure can in this sense be regarded as a driving force for cell enlargement even though the actual strain process and volume increase are caused by water uptake. However, an explicit treatment of the stress relaxation process that is basic to initiation of plant cell enlargement and the connexion between this process and the steady state strain phenomenon (equation (2)) seems not to have been given in the plant growth literature; Burström's note is helpful in drawing attention to this need, which we expect to be filled by future work.

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## Insecticide Resistance in Glasshouse Whitefly

DURING the autumn and winter of 1971 several nurserymen in south-east England reported failure to control glasshouse whitefly (*Trialeurodes vaporariorum* Westw.) with malathion and parathion at recommended rates of application; this suggests the possibility of chemical resistance to organophosphorus insecticides. Krause<sup>1</sup> has since reported similar suspicions of resistance in the Netherlands, but no experimental evidence has been published.

In preliminary observations we found that French beans (*Phaseolus vulgaris* L.) were suitable host plants for insecticide tests and that, using a whitefly population known to be susceptible to malathion, the first and second instar larval stages were the most susceptible in the insect. In the main trials six whitefly populations from south-east England (A-F in tables) were tested for resistance to malathion by dipping leaves infested with first instar larvae in aqueous emulsions at concentrations ranging from 2–3,910 p.p.m. malathion. 'Agral' (90% alkyl phenol ethylene oxide) wetting agent was used at 122 p.p.m. to ensure adequate wetting; control batches were dipped in wetting agent and water only. The five populations (A–E) and a population from eastern England (G) were also tested for DDT resistance, using concentrations of DDT of 8–4,725 p.p.m. Mortality was assessed five days after treatment.

**Table 1** Response of Six Whitefly Populations to Malathion, 1972

| Population | Slope $\pm$ s.e. | Log LC <sub>50</sub> $\pm$ s.e. | LC <sub>50</sub> (p.p.m.) |
|------------|------------------|---------------------------------|---------------------------|
| A          | 1.86 $\pm$ 0.187 | 1.39 $\pm$ 0.053                | 25                        |
| B          | 1.41 $\pm$ 0.199 | 2.15 $\pm$ 0.066                | 141                       |
| C          | 1.38 $\pm$ 0.239 | 2.40 $\pm$ 0.085                | 251                       |
| D          | 1.29 $\pm$ 0.182 | 2.78 $\pm$ 0.085                | 603                       |
| E          | 1.60 $\pm$ 0.156 | 2.89 $\pm$ 0.042                | 776                       |
| F          | 0.71 $\pm$ 0.331 | 3.41 $\pm$ 0.439                | 2,570                     |

When the probit mortalities in each population were plotted against log doses of each insecticide, no direct relationship was obtained (chi-square test;  $P < 0.05$  in each case). Tables 1–3 show the slope and concentration for 50% mortality (LC<sub>50</sub>) derived from the best-fitting line for each set of points. Population A was highly susceptible to malathion. The LC<sub>50</sub>s of the other five populations (B–F) were significantly greater than the LC<sub>50</sub> of population A ( $t$ -test;  $P < 0.05$ ). Estimated resistance levels varied from 6–100 times (B and F respectively) that of population A. Populations D and E were from separate glasshouses on the same holding and gave similar resistance levels. Population C was much more resistant to DDT than the other five populations. There was no apparent link between malathion resistance and DDT resistance.

In further tests we compared the susceptibilities of malathion-resistant populations D and E mixed together, with malathion-susceptible population A to dichlorvos,

**Table 2** Response of Six Whitefly Populations to DDT, 1972

| Population | Slope $\pm$ s.e. | Log LC <sub>50</sub> $\pm$ s.e. | LC <sub>50</sub> (p.p.m.) |
|------------|------------------|---------------------------------|---------------------------|
| B          | 1.60 $\pm$ 0.321 | 1.29 $\pm$ 0.151                | 19                        |
| G          | 1.39 $\pm$ 0.243 | 1.30 $\pm$ 0.135                | 20                        |
| D          | 1.61 $\pm$ 0.104 | 1.47 $\pm$ 0.036                | 30                        |
| E          | 1.43 $\pm$ 0.147 | 1.62 $\pm$ 0.054                | 42                        |
| A          | 4.44 $\pm$ 3.380 | 2.04 $\pm$ 0.290                | 110                       |
| C          | 0.79 $\pm$ 0.097 | 3.36 $\pm$ 0.101                | 2,291                     |



another organophosphorus insecticide. Populations D and E had been, respectively, 24 and 31 times as resistant to malathion as population A, and the mixed population proved to be 15 times more resistant to dichlorvos than population A.

**Table 3** Response of Two Whitefly Populations to Dichlorvos, 1972

| Population | Slope $\pm$ s.e. | Log LC <sub>50</sub> $\pm$ s.e. | LC <sub>50</sub> (p.p.m.) |
|------------|------------------|---------------------------------|---------------------------|
| A          | 1.44 $\pm$ 0.162 | 1.53 $\pm$ 0.058                | 34                        |
| D/E        | 0.90 $\pm$ 0.138 | 2.71 $\pm$ 0.106                | 513                       |

Our observations show that resistance to at least three chemicals occurs in glasshouse whitefly and that the level of resistance varies in different populations. It seems likely that resistance will become widespread through the distribution of plants infested with resistant whiteflies. Work is continuing on the assessment of other chemicals for the control of this pest.

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## Selective Feeding of *Anopheles gambiae* according to ABO Blood Group Status

IN investigations of physiological factors affecting the selection of human hosts for feeding by the mosquito *Anopheles gambiae* (species A from Nkolmekok<sup>2</sup>, Cameroons), pairs of subjects were chosen who contrasted in ABO blood group status. The experiments were conducted under conditions which simulated as closely as possible those under which females of *A. gambiae* normally bite. Because illumination was limited to a dim red light, counting how many of the twenty female insects used took a blood meal from the arm of a particular host proved difficult. We found, however, that even if only a very small blood meal was taken during the ten minutes' testing time, the ABO status of this meal could be reliably established within an hour, by extracting the blood from the mosquito's gut and performing direct agglutination tests on it with anti-A and anti-B sera. In over a hundred blind tests there were only two cases of possible error.

**Table 1** Mean Number of Bites in Each Group

| Blood group | No. of subjects | Mean No. of bites* |
|-------------|-----------------|--------------------|
| O           | 42              | 5.045              |
| A           | 41              | 3.276              |
| B           | 14              | 4.250              |
| AB          | 5               | 3.280              |
| A+B+AB      | 60              | 3.503              |

\* For each subject exposed more than once the average value was used.

Analysis of the blood group data reveals that the mosquitoes preferentially selected hosts of blood group O. The mean numbers of bites in the different groups are shown in Table 1. For statistical analysis the ratio of bites in individuals of one blood group to those of another group were used. The standardized normal deviate (s.n.d.) is calculated for each test, with continuity correction. For individuals of O and A blood

group (the most common test situation) this is expressed as

$$\log_e [(r_O + \frac{1}{2}) / (r_A + \frac{1}{2})] \\ \text{s.n.d.} = [(r_A + \frac{1}{2})^{-1} + (r_O + \frac{1}{2})^{-1}]^{\frac{1}{2}}$$

where  $r_O$  is the number of mosquitoes biting the O subject and  $r_A$  the number biting the A subject. This procedure obviates the variation in total number of mosquitoes choosing to feed from test to test and the logit transformation effectively normalizes the distribution of the ratio as is indicated by a normal plot of the s.n.d.s. Some subjects took part in several experiments in different combinations of pairs, but singling out for analysis only those cases where a subject was first used produces a normal plot with the same mean and slope as when using all the experiments, and this slope is very close to the theoretical value. All the variation is therefore accounted for by the blood group difference. On the null hypothesis that mosquitoes feed randomly with respect to ABO status, the s.n.d.s have a mean of 0 and the standard deviation of the mean equals  $1/n^{\frac{1}{2}}$ . In the 97 pairs of O and A individuals in these experiments, the sum of the s.n.d.s is 38.3 and  $t_{96}$  equals 3.9 which is significant at the 0.1% probability level. In O and B pairs  $t_{25}=3.3$ , significant at the 0.5% probability level. When O subjects are compared with all non-O subjects, the sum of the s.n.d.s is 59.2 and  $t_{135}=5.1$ , also significant at the 0.1% probability level.

These experiments strongly suggest that *A. gambiae* recognizes ABO blood group variation, and is selective in its feeding, with a preference for blood group O. The basis for this recognition and selection is not obvious, although ABH blood group substances do occur on skin cells<sup>1-3</sup> and have been reported in sweat<sup>4</sup>.

This finding could be of great importance in infectivity rates, the development of acquired immunity and natural selection for ABO blood groups in malarious regions. It needs, however, to be confirmed and extended to other strains of *A. gambiae* and other Anophelines.

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## Carcinogenic Nitrosamines formed by Drug/Nitrite Interactions

NITROSAMINES are compounds with broad carcinogenic activity in many animal species, but have seldom been detected in the environment in significant quantities. Man may nevertheless be exposed to nitrosamines formed *in vivo* from ingested nitrite and secondary amines<sup>1-3</sup>. Nitrite is a common constituent of the diet, as are several secondary amines<sup>4</sup>. Since tertiary amines can also react with nitrite<sup>5</sup> in the mildly acid conditions of the

mammalian stomach, the exposure of man to the many tertiary nitrogen compounds which are drugs<sup>6</sup> must also be considered.

We have examined the interaction of nitrite with six tertiary nitrogen compounds representative of different types of chemical structure: oxytetracycline (antibiotic), aminopyrine (analgesic), disulphiram (antialcoholic), nikethamide (respiratory stimulant), tolazamide (oral hypoglycaemic), and piperine (found in pepper), all of which gave measurable yields of nitrosamine.

We did not study these reactions exhaustively, but examined formation of nitrosamine products in varying conditions. Reactions were carried out in aqueous acid solutions with an excess of sodium nitrite, usually at 37° C and at a pH between 3.5 and 5.5. Reactions were terminated after 4 h or less by addition of KOH to pH 11, and the nitrosamines were extracted with methylene chloride and estimated by gas chromatography on columns of diethyleneglycol succinate on 'Chromosorb' W. The identity of the nitrosamine formed was established by mass spectrometry and in some cases by infrared spectrometry. The reaction conditions and yields of nitrosamines are given in Table 1.

All compounds tested gave measurable yields of nitrosamines at body temperature. The two tertiary amines, oxytetracycline and aminopyrine, gave high yields of dimethylnitrosamine (DMN) and were studied in more detail. In the aminopyrine reactions, the effect on nitrosamine formation of reactant concentration and pH is shown in Table 2. It appeared that DMN was formed in good yields over a wide range of pH, and that a large excess of nitrite did not increase the yield. The death from acute DMN hepatotoxicity of rats given small doses of aminopyrine and nitrite by stomach tube has been reported<sup>7</sup>.

Similar studies with oxytetracycline, however, showed a marked dependence of DMN yield on pH and on nitrite concentration (Table 3). The optimum pH was 3, and a lower yield of DMN at lower molar ratios of nitrite was found.

The dialkylamides, nikethamide, piperine, and disulphiram gave rather lower yields of the respective nitrosamines, diethylnitrosamine (DEN) and nitrosopiperidine (NP), than did the two tertiary amines. With these compounds as well, formation of nitrosamine was favoured at pH greater than 3, the yield of nitrosamine being quite small near pH 2. In the case of piperine and disulphiram, the percentage yield of nitrosamine might have been greater than indicated, because much of the starting material remained undissolved.

Tolazamide, which is both a substituted urea and a dialkylhydrazine, was converted readily to N-nitrosohexamethylenimine (NHM), which has been reported as a potent liver carcinogen in rats<sup>8</sup>. The reaction of N',N'-dialkylhydrazides with nitrous acid was studied<sup>9</sup>, but nitrosamines were not positively identified in the products.

Although these reactions were conducted with higher concentrations of drug and nitrite than would be found in the human stomach, the yields of nitrosamine expected in normal use of the drugs may still be predicted from the course of the reactions. In the case of aminopyrine, a large proportion of the amine is converted to DMN regardless of concentration. For example, a dose of 100 mg would be expected to yield several mg of DMN, a significant amount of such a potent carcinogen. The reaction of disulphiram and tolazamide with nitrite is so rapid that mg quantities of nitrosamine would be expected, in the presence of sufficient nitrite. The reaction of oxytetracycline, however, is markedly dependent on concentration and pH and the amount of DMN produced might well be negligible

Table 1 Reaction of Drugs with Nitrite

| Compound        | Wt.    | Nitrite | Acid                                   | Vol.   | pH  | Temp. | Time | Yield of nitrosamine |
|-----------------|--------|---------|----------------------------------------|--------|-----|-------|------|----------------------|
| Oxytetracycline | 250 mg | 0.5 g   | 1 ml. AcOH                             | 12 ml. | 4.0 | 37° C | 4 h  | 24 mg DMN (65%)      |
|                 | 250 mg | 0.5 g   | 2.3 g NaH <sub>2</sub> PO <sub>4</sub> | 12 ml. | 4.2 | 37° C | 4 h  | 4.2 mg DMN (11%)     |
| Aminopyrine     | 1.0 g  | 2.0 g   | 4 ml. AcOH                             | 25 ml. | 4.2 | 37° C | 4 h  | 234 mg DMN (73%)     |
|                 | 1.0 g  | 2.0 g   | 8.6 g NaH <sub>2</sub> PO <sub>4</sub> | 25 ml. | 4.7 | 37° C | 4 h  | 106 mg DMN (33%)     |
| Disulphiram     | 0.74 g | 1.8 g   | 1.5 ml. AcOH                           | 12 ml. | 4.0 | 37° C | 2 h  | 22 mg DEN (4.4%)     |
|                 | 0.74 g | 1.8 g   | 3.4 g NaH <sub>2</sub> PO <sub>4</sub> | 12 ml. | 4.6 | 37° C | 2 h  | 31 mg DEN (6.2%)     |
| Nikethamide     | 0.74 g | 1.8 g   | 0.01 M HCl                             | 12 ml. | 2.0 | 37° C | 2 h  | 3 mg DEN (0.6%)      |
|                 | 1.0 g  | 1.7 g   | 1.5 ml. AcOH                           | 12 ml. | 3.8 | 37° C | 2 h  | 0.8 mg DEN (0.13%)   |
|                 | 1.0 g  | 1.7 g   | 0.01 M HCl                             | 12 ml. | 2.1 | 37° C | 20 h | 0.02 mg DEN (0.003%) |
|                 | 1.0 g  | 1.7 g   | 0.01 M HCl                             | 12 ml. | 3.0 | 37° C | 20 h | 0.06 mg DEN (0.01%)  |
|                 | 1.0 g  | 1.7 g   | 0.01 M HCl                             | 12 ml. | 3.6 | 37° C | 20 h | 1.65 mg DEN (0.28%)  |
|                 | 1.0 g  | 1.7 g   | 0.01 M HCl                             | 12 ml. | 4.2 | 37° C | 20 h | 1.65 mg DEN (0.28%)  |
| Tolazamide      | 4.0 g  | 3.5 g   | 3 ml. AcOH                             | 25 ml. | 4.4 | 37° C | 4 h  | 445 mg NHM (28%)     |
|                 | 4.0 g  | 3.5 g   | 8.6 g NaH <sub>2</sub> PO <sub>4</sub> | 25 ml. | 4.2 | 37° C | 4 h  | 367 mg NHM (23%)     |
| Piperine        | 100 mg | 1.0 g   | 1.5 ml. AcOH                           | 12 ml. | 3.6 | 90° C | 4 h  | 11.2 mg NP (27%)     |
|                 | 100 mg | 1.0 g   | 1.5 ml. AcOH                           | 12 ml. | 3.6 | 37° C | 4 h  | 2.2 mg NP (5%)       |
|                 | 100 mg | 1.0 g   | 0.01 M HCl                             | 12 ml. | 2.3 | 37° C | 4 h  | 0.05 NP (0.1%)       |

Table 2 Reaction of Aminopyrine with Nitrite

| Wt. of aminopyrine | Vol.   | Wt. of NaNO <sub>2</sub> | Acid                                                | pH  | Temp. | Time | Yield of DMN |
|--------------------|--------|--------------------------|-----------------------------------------------------|-----|-------|------|--------------|
| 1.0 g              | 25 ml. | 2.0 g (6 mol)            | 4 ml. AcOH                                          | 4.2 | 37° C | 4 h  | 234 mg (73%) |
| 1.0 g              | 25 ml. | 2.0 g (6 mol)            | 8.6 g NaH <sub>2</sub> PO <sub>4</sub>              | 4.7 | 37° C | 4 h  | 106 mg (33%) |
| 1.0 g              | 25 ml. | 2.0 g (6 mol)            | 8.6 g NaH <sub>2</sub> PO <sub>4</sub> + 3.7 g NaCl | 4.2 | 37° C | 4 h  | 109 mg (34%) |
| 0.36 g             | 12 ml. | 0.42 g (4 mol)           | HCl                                                 | 2.5 | 37° C | 3 h  | 66 mg (57%)  |
| 0.36 g             | 12 ml. | 0.42 g (4 mol)           | HCl                                                 | 3.2 | 37° C | 3 h  | 77 mg (67%)  |
| 0.36 g             | 12 ml. | 0.42 g (4 mol)           | HCl                                                 | 3.9 | 37° C | 3 h  | 61 mg (53%)  |
| 0.36 g             | 12 ml. | 0.42 g (4 mol)           | HCl                                                 | 4.6 | 37° C | 3 h  | 45 mg (39%)  |
| 62 mg              | 10 ml. | 56 mg (3 mol)            | 0.01 M HCl                                          | 5.3 | 37° C | 3 h  | 6.6 mg (33%) |
| 62 mg              | 10 ml. | 56 mg (3 mol)            | 0.01 M HCl + 0.12 ml. AcOH                          | 3.5 | 37° C | 3 h  | 7.0 mg (35%) |
| 62 mg              | 10 ml. | 100 mg (5 mol)           | 0.01 M HCl                                          | 5.5 | 37° C | 3 h  | 6.2 mg (31%) |
| 62 mg              | 10 ml. | 100 mg (5 mol)           | 0.01 M HCl + 0.12 ml. AcOH                          | 3.5 | 37° C | 3 h  | 6.9 mg (35%) |
| 62 mg              | 10 ml. | 200 mg (10 mol)          | 0.01 M HCl                                          | 5.7 | 37° C | 3 h  | 6.8 mg (34%) |
| 62 mg              | 10 ml. | 200 mg (10 mol)          | 0.01 M HCl + 0.12 ml. AcOH                          | 3.7 | 37° C | 3 h  | 7.1 mg (36%) |



**Table 3** Reaction of Oxytetracycline with Nitrite

| Wt. of OTC | Vol.   | Wt. of NaNO <sub>2</sub> | Acid                                                   | pH  | Temp. | Time  | Yield of DMN   |
|------------|--------|--------------------------|--------------------------------------------------------|-----|-------|-------|----------------|
| 500 mg     | 20 ml. | 1.0 g                    | 3 ml. AcOH                                             | 4.2 | 90° C | 16 h  | 74 mg (100%)   |
| 250 mg     | 10 ml. | 0.5 g                    | 1 ml. AcOH                                             | 3.5 | 37° C | 4 h   | 24 mg (65%)    |
| 250 mg     | 12 ml. | 0.5 g                    | 2.3 g NaH <sub>2</sub> PO <sub>4</sub>                 | 4.2 | 37° C | 4 h   | 4.2 mg (11%)   |
| 250 mg     | 12 ml. | 0.5 g                    | 2.3 g NaH <sub>2</sub> PO <sub>4</sub><br>+ 2.5 g NaCl | 3.7 | 37° C | 4 h   | 7.4 mg (20%)   |
| 100 mg     | 4 ml.  | 56 mg                    | 0.05 ml. AcOH                                          | 3.6 | 37° C | 1 h   | 0.9 mg (6%)    |
| 100 mg     | 4 ml.  | 56 mg                    | 0.05 ml. AcOH                                          | 3.6 | 37° C | 2 h   | 1.9 mg (13%)   |
| 100 mg     | 4 ml.  | 56 mg                    | 0.05 ml. AcOH                                          | 3.6 | 37° C | 2.5 h | 2.6 mg (18%)   |
| 100 mg     | 12 ml. | 56 mg                    | HCl                                                    | 2.0 | 37° C | 4 h   | 0.03 mg (0.2%) |
| 100 mg     | 12 ml. | 56 mg                    | HCl                                                    | 3.0 | 37° C | 4 h   | 0.3 mg (2.0%)  |
| 100 mg     | 12 ml. | 56 mg                    | HCl                                                    | 4.0 | 37° C | 4 h   | 0.03 mg (0.2%) |
| 100 mg     | 12 ml. | 200 mg                   | HCl                                                    | 2.0 | 37° C | 4 h   | 0.1 mg (0.7%)  |
| 100 mg     | 12 ml. | 200 mg                   | HCl                                                    | 3.0 | 37° C | 4 h   | 2.2 mg (15%)   |
| 100 mg     | 12 ml. | 200 mg                   | HCl                                                    | 4.0 | 37° C | 4 h   | 1.0 mg (7%)    |

The hazard of nitrosamine formation from various drugs is therefore variable, but no exposure to a carcinogen can be considered insignificant.

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## Mechanism of the Antitumour Effect of Interferon in Mice

REPEATED inoculation of mouse interferon preparations inhibits the growth of several murine transplantable tumours and increases the survival of tumour inoculated mice<sup>1-6</sup>. The mechanism of this antitumour action of interferon *in vivo* is unknown. Since comparable interferon preparations inhibited the division of murine leukaemia L 1210 and Ehrlich ascites (EA) cells in suspension cultures<sup>7-9</sup> it is possible that interferon also acts *in vivo* by inhibiting tumour cell multiplication. It is also possible, however, that interferon enhances in some manner the capacity of the host to reject the transplanted tumour.

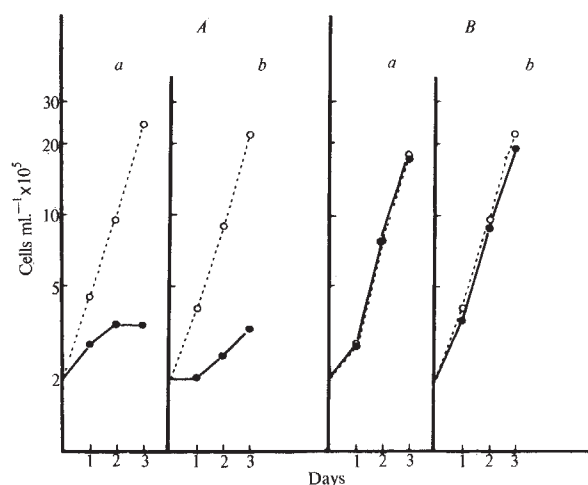
We have used murine leukaemia L 1210 cells which are sensitive to the antiviral effect of interferon and to the inhibitory effect of interferon on cell division<sup>7-9</sup>. We refer to these cells as L 1210 S cells to distinguish them from cells of a mutant cell subline, L 1210 R, resistant to both the antiviral and cell division inhibitory activities of interferon<sup>8,9</sup>.

We reasoned that if the antitumour effect resulted from a direct inhibition of the multiplication of tumour cells by inter-

feron, interferon would protect mice inoculated with interferon-sensitive cells but would not protect mice inoculated with interferon-resistant cells. On the other hand if the antitumour effect of interferon were in part host mediated, interferon might protect mice inoculated either with interferon-sensitive or interferon-resistant cells.

We therefore determined the effect of interferon treatment on DBA/2 mice inoculated with interferon-sensitive or interferon-resistant L 1210 cells. The origins of L 1210 S and L 1210 R cells and the techniques of cultivation *in vitro* have been previously described<sup>7-9</sup>. One to two-month-old male or female DBA/2 mice were inoculated intraperitoneally with either L 1210 S or L 1210 R cells. Twenty-four hours later, mice of each group were inoculated with 50,000-64,000 NIH international reference units of mouse brain or cell culture interferon, and treatment was continued once daily for one month. Control mice were inoculated with a mock interferon or left untreated. The techniques used in the preparation and assay of concentrated mouse brain or cell culture interferon have been previously described<sup>2,5,8,9</sup>.

Daily interferon treatment was associated with an increase in the mean survival time of DBA/2 mice inoculated with interferon-sensitive and with interferon-resistant L 1210 cells (Table 1). A protective effect was observed in mice treated with mouse interferon derived from brain or from cell culture medium, and in mice inoculated with varying numbers of tumour cells (Table 1). There was no protection in mice treated with control preparations or with a heterologous



**Fig. 1** Effect of interferon on the multiplication *in vitro* of L 1210 S (A) or L 1210 R (B) cells obtained from the peritoneal cavities of DBA/2 mice. a, Control; b, interferon treated. "Interferon treated" refers to the treatment of a mouse inoculated with L 1210 S or R cells. Cells cultivated in non-agitated suspension culture: ○, no interferon; ●, 1,600 units mouse cell culture interferon/0.2 ml.

**Table 1** Effect of Interferon Treatment on Mice inoculated with Interferon Sensitive or Interferon Resistant L 1210 Cells

| Experiment No. | No. of L 1210 cells inoculated in each mouse | Treatment               | Mice inoculated with                                 |                           |                          |                                                      |                           |                          |
|----------------|----------------------------------------------|-------------------------|------------------------------------------------------|---------------------------|--------------------------|------------------------------------------------------|---------------------------|--------------------------|
|                |                                              |                         | Interferon-sensitive L 1210 S cells                  |                           |                          | Interferon-resistant L 1210 R cells                  |                           |                          |
|                |                                              |                         | No. of mice surviving > 60 days/total No. inoculated | Mean No. of days survival | Confidence interval 0.95 | No. of mice surviving > 60 days/total No. inoculated | Mean No. of days survival | Confidence interval 0.95 |
| 1              | 10 <sup>4</sup>                              | Control preparation     | 0/9                                                  | 28 *                      | 27-30                    | 0/9                                                  | 25 *                      | 23-27                    |
|                |                                              | Brain interferon        | 0/9                                                  | 38 *                      | 33-44                    | 0/7                                                  | 31 *                      | 27-35                    |
|                | 10 <sup>3</sup>                              | Control preparation     | 0/10                                                 | 30 *                      | 29-32                    | 0/9                                                  | 28 †                      | 26-31                    |
|                |                                              | Brain interferon        | 0/10                                                 | 41 *                      | 38-46                    | 0/9                                                  | 33 †                      | 30-38                    |
|                | 10 <sup>2</sup>                              | Control preparation     | 0/8                                                  | 34 *                      | 31-37                    | 0/8                                                  | 29 †                      | 27-31                    |
|                |                                              | Brain interferon        | 8/9                                                  | — *                       | —                        | 2/9                                                  | 45 †                      | 31-81                    |
| 2              | 10 <sup>4</sup>                              | None                    | 0/16                                                 | 25 *                      | 23-27                    | 0/16                                                 | 23 *                      | 22-24                    |
|                |                                              | Cell culture interferon | 0/16                                                 | 38 *                      | 36-41                    | 0/16                                                 | 36                        | 34-39                    |

\*  $P < 0.001$ . †  $P < 0.01$ .

rabbit serum interferon of high titre. The results of three experiments (experiment 1, Table 1) suggested that interferon treatment gave a greater protective effect in mice inoculated with L 1210 S cells than in mice inoculated with L 1210 R cells.

There is strong evidence that the character of resistance to interferon did not change during *in vivo* passage, and that it was not influenced by interferon treatment. Four moribund DBA/2 mice were sacrificed 3-4 weeks after inoculation with 10<sup>4</sup> L 1210 cells. Two mice had been inoculated with L 1210 S cells and two mice with L 1210 R cells. One mouse in each group had received interferon daily for one month. L 1210 cells were harvested from the peritoneal cavity of each mouse and cultivated for one passage *in vitro* as previously described<sup>8,9</sup> before determining the effect of interferon on cell multiplication. The multiplication of L 1210 S cells derived from an untreated or an interferon-treated mouse was inhibited by interferon (Fig. 1). In contrast, the multiplication of L 1210 R cells derived from an untreated or an interferon-treated mouse was not inhibited by interferon.

Interferon in mice inoculated intraperitoneally with L 1210 cells may act directly on the tumour cells by inhibiting their multiplication on the host, enhancing mechanisms of tumour cell rejection, or on both tumour cells and host.

Our finding that interferon treatment increased the survival of mice inoculated with interferon-resistant L 1210 cells suggests that the antitumour effect in these mice did not result from a direct inhibitory effect on tumour cell multiplication. We suggest that in mice inoculated with L 1210 R cells interferon acted in some manner on the mouse and inhibition of tumour growth was mediated by the host. In mice inoculated with the parental L 1210 S cells, sensitive to interferon, both mechanisms may have been operative, that is a direct inhibitory effect on cell multiplication and a host mediated effect. This interpretation was supported by results which suggested a greater protective effect of interferon in mice inoculated with L 1210 S cells than in mice inoculated with L 1210 R cells. Work is in progress to determine the nature of the host mediated effect induced by interferon.

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## Differential Staining Patterns of Human Chromosomes treated with Potassium Permanganate

THE permanganate ion has occasionally been used in histology and histochemistry to demonstrate particular components in animal tissues<sup>1,2</sup>. In electron microscopy, potassium permanganate has been used in some fixatives<sup>3</sup> and as a stain<sup>4</sup>. Permanganate oxidation has also been used to chemically degrade DNA<sup>5</sup> and under certain mild conditions to oxidize preferentially pyrimidine residues, especially the thymine residue of heat-denatured DNA with a small strand break<sup>6,7</sup>.

During a study of modification of pyrimidine residues of DNA in cytological preparations with potassium permanganate, it was noticed that the metaphase chromosomes of humans, rats, mice, and Chinese hamsters exhibited clear banded patterns when stained with buffered Giemsa stain after permanganate treatment. The mechanism of differential staining after permanganate oxidation of the metaphase chromosomes was not clear, but our (unpublished) results suggested that oxidation of the thymine residues of chromosomal DNA might not be responsible.

Human leucocyte cultures with *Phytolacca americana* mitogen, HeLa cells, a suspension culture of a strain established from a human nasopharyngeal carcinoma, another suspension culture of rat leukaemia cells, L cells, and fibroblast cultures of Chinese hamsters were treated with Colcemid for 2 h. The cells were collected either by simple centrifugation or with trypsin. They were treated with 0.075 M potassium chloride for 15 min at room temperature, and fixed with three changes of a mixture of methanol and glacial acetic acid (3:1). Chromosome spreads were made on slides by air-drying without heat-treatment.

The slides were incubated for 20-40 min containing 10 mM potassium permanganate solution prepared in 33 mM phosphate buffer at pH 7.0 and containing 5 mM magnesium sulphate in an ice-bath. They were then washed in running

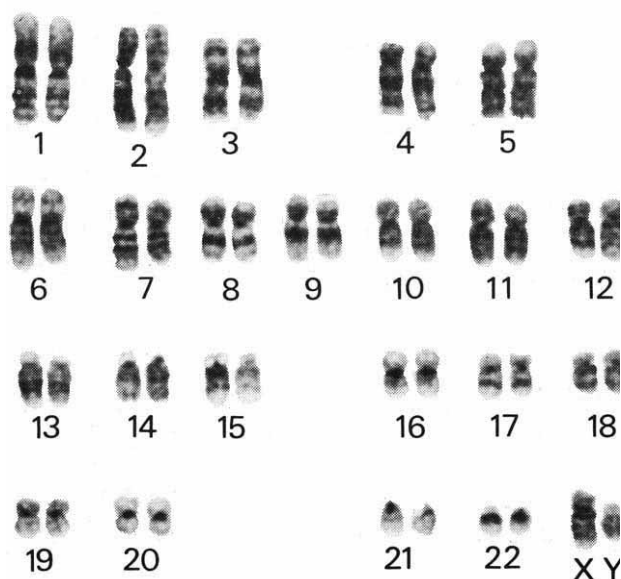


tap water for about 30 s, soaked in absolute ethanol for 5 min, and air-dried. Slides were stained for 5–10 min with Giemsa stain diluted approximately 100 times with phosphate buffer at pH 7.0. Preparations were sealed with Eukitt which contained 1% butyl hydroxy toluene as an antioxidant. Controls were treated with buffer solution alone before Giemsa staining.

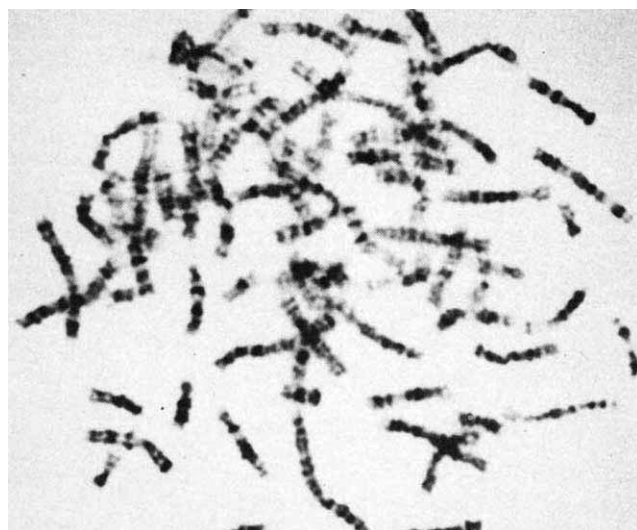
In human chromosomes, the permanganate treatment produced slightly swollen chromosomes with a banded appearance (Figs. 1 and 2), but controls did not show any banding. With excessive permanganate treatment only the centromeric regions of several homologous pairs stained. The differential staining patterns were easily reproduced, but overcontracted chromosomes tended to lose fine details. The late prophase to early metaphase chromosomes were suitable for minute analysis of the patterns and identification of individual chromosomes.

The patterns of differential staining along the longitudinal axis of the chromosomes seemed to be similar to those illustrated by other workers<sup>8–10</sup> in this field, but on close examination some differences in banding patterns were evident. Some examples are shown in Fig. 1: points of difference are: (i) Telomeric regions of both arms of Nos. 4, 7, and 9; of long arms of Nos. 2, 10, 13, 15, and 17; and of short arms of Nos. 1, 5, and 16 were easily swollen and stained only faintly. In contrast, the telomeric regions of the long arm of No. 14 and short arms of Nos. 2, 12, and 18 were consistently well defined and moderately stained. (ii) The proximal region of the short arm of No. 6 had a clear segment and appeared slightly elongated. (iii) The X chromosome had three dark bands on its long arm, a clear telomere, and one dark block close to the centromere on the short arm. The Y chromosome did not stain as heavily as the centromeric regions of some homologous pairs or the conspicuous bands in some of the A and C group chromosomes. In most metaphase cells the long arm of the Y chromosome stained moderately densely, and had a narrow clear band about one third of the length from the centromere. No telomeric swelling was observed.

The same procedures of permanganate treatment and of Giemsa staining were used for chromosome preparations of rats, mice, and Chinese hamsters, and the banding patterns were easily reproduced, and helped to identify individual chromosomes.



**Fig. 1** Normal human male karyotype. Chromosome spreads of the leucocyte culture were made by the standard air-drying method. The slide was treated with 10 mM  $\text{KMnO}_4$  solution at pH 7.0 and at 0° C for 30 min and stained with Giemsa stain. Conspicuous similarity in the banding patterns in each homologous pair may be noticed.



**Fig. 2** HeLa cell chromosomes at early metaphase. The air-dried preparation was treated as described for Fig. 1. Note numerous very delicate bands along chromosome axes.

Caspersson *et al.* have demonstrated differential affinity of chromosome segments along the longitudinal axis of a chromosome by the fluorescence technique<sup>11</sup>. The fluorescent bands seem to be correlated with the differential Giemsa staining patterns produced by various treatments<sup>18–20</sup>. The differential staining properties of mouse chromosomes by Giemsa stain were first observed by Pardue and Gall<sup>12</sup>, and Arrighi and Hsu located constitutive heterochromatin in human chromosomes using Giemsa staining<sup>13</sup>. Apart from so-called C-bands revealed by the Pardue–Gall and the Arrighi–Hsu methods, various procedures using Giemsa stain to reveal G-bands have been presented recently<sup>8–10,14–18</sup>. Many of these methods use rather high temperatures (about 60° C), alkaline pretreatment of slides<sup>8,9,15,17</sup>, or use proteolytic enzymes<sup>10</sup> which may cause denaturation of chromosomal DNA or remove the protein component from the chromosome. The permanganate treatment is at low temperatures and does not have these effects.

The banding patterns obtained by the method described are useful in the identification of individual chromosomes of various animals and of hybrid cells and in the analysis of chromosome aberrations; however, the relations of the banding patterns to the chemical organization of the chromosome and to the functional aspects of individual chromosome segments are yet to be elucidated.

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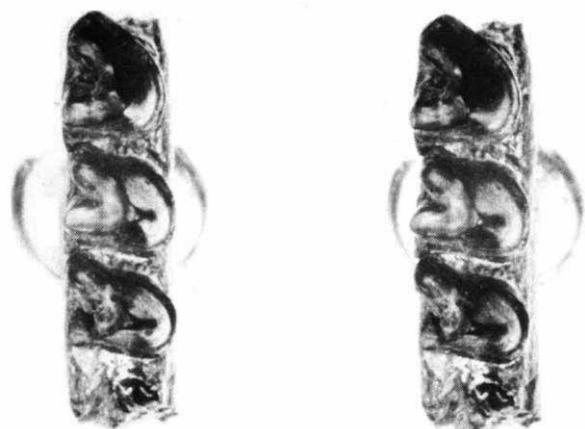
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**Fig. 1** Stereophoto pair of right mandibular fragment with three molariform teeth (University of Alberta catalogue number 8588), symmetrodont, Upper Milk River Formation, Alberta, Canada ( $\times c. 12$ ).

and those that are found are mostly fragmentary jaws with teeth. The geologically oldest symmetrodonts are from the Rhaetic of Wales (*Kuehneotherium*<sup>2</sup>); Upper Jurassic (*Spalacotherium*, *Tinodon*, *Eurylemba*, *Amphidon*) and Lower Cretaceous (*Spalacotherium*, *Spalacotheroides*, *Manchurodon*) symmetrodonts are known from Eurasia and North America<sup>3-7</sup>. Here I report the discovery of the first Upper Cretaceous symmetrodont, from the early Campanian<sup>8</sup> Upper Milk River Formation<sup>9</sup>, Alberta, Canada.

The Alberta fossil (Fig. 1) is a right mandibular fragment with three tricuspid molariform teeth resembling postcanine teeth of the spalacotheriid *Spalacotheroides bridwelli*<sup>6</sup>, from the Albian of Texas, United States. Particular resemblances include high protoconid, lower paraconid and metaconid, and a basal cingulum on each of the comparable teeth. On the Alberta fossil, the cusps of the posterior two teeth form acute trigonid angles as in *Spalacotheroides*; the crown of the most anterior tooth forms a more obtuse triangle, but this may be because the tooth has a relatively anterior position in the complete dental series. Detailed description, comparisons, and the naming of the Alberta symmetrodont will be published elsewhere.

In addition to the new symmetrodont, early mammals from the Upper Milk River Formation now include a triconodont<sup>10</sup>, diverse ptilodontoid and taeniolabidoid multituberculates<sup>11</sup>, didelphid, pedomioid and stagodontid marsupials<sup>12</sup>, a primitive therian of metatherian-eutherian grade<sup>13</sup> and a generalized eutherian<sup>14</sup>, the oldest discovered assemblage of Upper Cretaceous mammals from North America<sup>15</sup>. The temporally relict triconodont, symmetrodont, and therian of metatherian-eutherian grade document an archaic aspect of mammalian fauna of the Milk River Formation not previously known among Upper Cretaceous mammal assemblages from North America or elsewhere.

The mammalian fauna of the Upper Milk River Formation demonstrates that the evolutionary replacement of archaic mammal faunas characteristic of Upper Jurassic and Lower Cretaceous horizons (including, in sum, triconodonts, docodonts, plagiaulacoid multituberculates, symmetrodonts, eupantotheres, primitive therians of metatherian-eutherian grade, and ancestral marsupials and placentals) by progressive mammal faunas characteristic of uppermost Cretaceous horizons (including, in sum, advanced multituberculates, diverse opossum-like marsupials, insectivores and early insectivore derivatives) had not been completed, at least in North America, by middle Late Cretaceous time. Consequently, (1) the geological age of the Manchurian eutherian? *Endotherium*<sup>16</sup> is now uncertain, because the supposed Jurassic or Early Cretaceous age of the species is mostly based on its occurrence

in symmetrodont-bearing rocks<sup>17</sup>; and (2) a relatively great age<sup>17</sup> within the Upper Cretaceous of mammalian assemblages from the Gobi Desert, Mongolia<sup>18,19</sup>, is improbable, because these assemblages lack representatives of any of the diverse relict lineages of mammals now known to have survived to at least the early Campanian.

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## Copper and Zinc Status of Ewes and Lambs receiving Increased Dietary Concentrations of Cadmium

VEGETATION near some industrial complexes shows appreciable contamination with cadmium from certain airborne smokes and dusts. Goodman and Roberts<sup>1</sup> found concentrations up to 40 p.p.m. Cd in *Festuca* spp. growing in areas exposed to industrial contamination in a restricted area in South Wales. Samples from areas not subject to such contamination contained only 0.8 p.p.m. We analysed mixed herbage from an area in south-west England up to 3 miles downwind from a (presumed) source of airborne Cd contaminant. Winter herbage contained concentrations up to 40 p.p.m. with an overall annual mean of 10.1 p.p.m.

Experiments with rats<sup>2</sup>, chicks<sup>3</sup>, goats and pigs<sup>4</sup> show that high dietary Cd concentrations (75–100 p.p.m. Cd) have adverse effects on liver copper storage, growth, blood haemoglobin concentration and reproductive performance. Many of these effects were prevented by simultaneously increasing the Cu content of the diets, which suggest that Cd is a powerful metabolic antagonist of Cu. Further evidence suggests an additional antagonistic effect of Cd on zinc utilization<sup>5</sup>.

Our object was to determine whether dietary Cd concentrations within the range found in contaminated herbage had detectable effects on Cu and Zn metabolism in sheep during late pregnancy and early lactation.

Twenty-four autumn-tupped Cheviot ewes were randomized according to weight into four treatment groups during the 13th or 14th week of pregnancy, and given a diet of dried grass cubes containing crude protein ( $N \times 6.25$ ), 11.7%; Cd, 0.7 p.p.m.; Cu, 4.9 p.p.m.; Mo, 0.3 p.p.m.; and Zn, 37 p.p.m.

Cd intake was adjusted by spraying solutions of cadmium sulphate onto layers of grass cubes. The final Cd concentration (p.p.m.) of the diets given was as follows: group A, 0.7 (no Cd supplement); group B, 3.5; group C, 7.1; and group D, 12.3. All animals had unrestricted access to deionized water.

Nineteen one-day-old lambs selected at random from ewes bearing twins or triplets were sacrificed to determine concentrations of tissue Cd, Cu, Zn and Fe at birth. The remaining 20 lambs were housed with their dams and had access to the dam's diet. These lambs and four ewes from each group were slaughtered for tissue analysis when the lambs were 7.5–8 weeks old. Concentrations of whole blood Cu and Cd and plasma Zn and Cd were determined by atomic absorption spectrophotometry. Caeruloplasmin was determined by the method of Houchin<sup>6</sup> but using Bandrowski's base as external standard<sup>7</sup>. Zn, Cu and Cd analyses of ewe and lamb organs were carried out by atomic absorption analysis after acid digestion of freeze-dried tissues. Iron was determined as the Fe-tripridyltriazine complex in a 'Technicon' autoanalyser<sup>8</sup>. Statistical analysis of liver and kidney data was carried out after logarithmic transformation of individual values.

Cadmium supplementation during the last third of pregnancy and during early lactation had only small and statistically non-significant effects in depressing plasma Zn, caeruloplasmin and

whole blood Cu in ewes, and had no influence on whole blood or plasma Cd concentrations. In contrast, Cd administration substantially decreased whole blood Cu and caeruloplasmin concentrations in lambs by the end of the experiment and had a similar but lesser effect on plasma Zn (Table 1). Whole blood and plasma Cd concentrations did not differ greatly between groups.

Of the 15 lambs originating from Cd supplemented groups, 12 had whole blood Cu concentrations below 0.6 µg/ml.; none of the 5 lambs from the control group (A) fell into this category. It has been suggested that levels of blood Cu below 0.6 µg/ml. in sheep or cattle are indicative of Cu deficiency<sup>9</sup>. Appreciating that this is a broad generalization because of individual differences in relationships between blood Cu concentration and clinical condition, the effects on whole blood Cu and caeruloplasmin nevertheless indicate a significant derangement of Cu metabolism.

Cadmium administration also depressed plasma Zn concentrations, but not to the levels encountered in lambs showing clinical signs of Zn deficiency (<0.3 µg/ml.)<sup>10</sup>. Cd depression of whole blood Zn in calves given diets containing 160 p.p.m. Cd has been reported<sup>5</sup>.

Table 2 summarizes analytical results on the livers of experimental animals. Exposure to diets supplemented with Cd resulted in highly significant increases in liver Cd concentration in ewes and lambs killed at the termination of the experiment. No treatment effects on liver Cd were noted in the lambs killed at birth, suggesting that the placenta may provide an effective barrier to Cd transport during foetal development. Since the Cd content of the milk of ewes was not influenced

**Table 1** Blood Cd, Zn, Cu, and Caeruloplasmin in Lambs at Termination of Experiment

| Dietary Cd concentration (p.p.m.)               | 0.7           | 3.5         | 7.1         | 12.3        | Significance of treatment effects |
|-------------------------------------------------|---------------|-------------|-------------|-------------|-----------------------------------|
| Number of lambs sampled                         | 5             | 5           | 4           | 6           |                                   |
| Whole blood Cd (µg ml. <sup>-1</sup> )          | 0.17 ± 0.01 * | 0.17 ± 0.02 | 0.17 ± 0.07 | 0.19 ± 0.01 | N.s. †                            |
| Plasma Cd (µg ml. <sup>-1</sup> )               | 0.21 ± 0.02   | 0.20 ± 0.02 | 0.18 ± 0.01 | 0.20 ± 0.01 | N.s.                              |
| Plasma Zn (µg ml. <sup>-1</sup> )               | 1.08 ± 0.06   | 1.03 ± 0.08 | 0.97 ± 0.01 | 0.75 ± 0.06 | P < 0.05                          |
| Whole blood Cu (µg ml. <sup>-1</sup> )          | 0.84 ± 0.13   | 0.56 ± 0.03 | 0.63 ± 0.03 | 0.44 ± 0.03 | P < 0.01                          |
| Caeruloplasmin activity (IU ml. <sup>-1</sup> ) | 27 ± 4        | 18 ± 4      | 22 ± 4      | 15 ± 1      | P < 0.05                          |

\* Standard error of mean. † N.s., not statistically significant ( $P > 0.05$ ).

**Table 2** Trace Metal Content (p.p.m. d.m.) of Livers of Ewes and Lambs given Diets differing in Cadmium Content during Late Pregnancy and Early Lactation

| Cadmium content of diet (p.p.m. d.m.)            | 0.7           | 3.5         | 7.1         | 12.3         | Significance of treatment effects |
|--------------------------------------------------|---------------|-------------|-------------|--------------|-----------------------------------|
| <b>Ewe liver (at termination of experiment)</b>  |               |             |             |              |                                   |
| Cadmium                                          | 0.95 ± 0.37 * | 2.01 ± 0.27 | 3.50 ± 0.95 | 11.20 ± 1.17 | P < 0.001                         |
| Copper                                           | 185 ± 51      | 174 ± 32    | 131 ± 56    | 59 ± 19      | P < 0.05                          |
| Zinc                                             | 109 ± 3       | 106 ± 11    | 107 ± 4     | 114 ± 7      | N.s.                              |
| Number of livers analysed                        | 4             | 4           | 4           | 4            |                                   |
| <b>Lamb liver (at termination of experiment)</b> |               |             |             |              |                                   |
| Cadmium                                          | 0.14 ± 0.01   | 1.57 ± 0.48 | 3.49 ± 1.36 | 10.95 ± 1.00 | P < 0.001                         |
| Copper                                           | 101 ± 31      | 22 ± 3      | 62 ± 35     | 13 ± 2       | P < 0.01                          |
| Zinc                                             | 135 ± 8       | 96 ± 5      | 103 ± 3     | 94 ± 6       | P < 0.01                          |
| Number of livers analysed                        | 5             | 5           | 4           | 6            |                                   |
| <b>Lamb liver (at birth)</b>                     |               |             |             |              |                                   |
| Cadmium                                          | < 0.14        | < 0.14      | < 0.14      | < 0.14       | N.s.                              |
| Copper                                           | 183 ± 91      | 213 ± 47    | 115 ± 27    | 73 ± 38      | N.s.                              |
| Zinc                                             | 178 ± 81      | 266 ± 72    | 137 ± 25    | 148 ± 55     | (0.1 > P > 0.05)                  |
| Number of livers analysed                        | 3             | 3           | 8           | 5            | N.s.                              |

\* Standard error of mean. † N.s., not statistically significant ( $P > 0.05$ ).



**Table 3** Concentrations of Cd, Zn, and Cu (p.p.m. d.m.) in Mixed Pasture Herbage

| (i) From agricultural land exposed to contamination from industrial sources     |         |             |       |                   |                                                             |
|---------------------------------------------------------------------------------|---------|-------------|-------|-------------------|-------------------------------------------------------------|
| Season                                                                          | Cd      | Zn          | Cu    | Number of samples | Remarks                                                     |
| Winter                                                                          | 6       | 650         | 17    | 1                 | Sampled 3 miles downwind of possible source *               |
| Summer                                                                          | 1-2     | 160-200     | 8-13  | 3                 |                                                             |
| Winter                                                                          | 13-14   | 1,100-1,320 | 38-48 | 2                 | Sampled 2 miles downwind of possible source                 |
| Summer                                                                          | 2-4     | 210-370     | 8-21  | 2                 |                                                             |
| Winter                                                                          | 21-41   | 1,710-3,140 | 44-95 | 2                 | Sampled 1 mile downwind of possible source *                |
| Summer                                                                          | 5-10    | 510-710     | 15-19 | 2                 |                                                             |
| Winter                                                                          | 4-17    | 210-510     | —     | —                 | 82 samples from 8 farms in vicinity of industrial complex † |
| Spring                                                                          | 2-15    | 155-440     | —     | —                 |                                                             |
| Summer                                                                          | 2-8     | 90-360      | —     | —                 |                                                             |
| (ii) Typical ranges of concentrations in herbage remote from industrial areas * |         |             |       |                   |                                                             |
|                                                                                 | 0.1-0.8 | 25-35       | 5-10  |                   |                                                             |

\* Analyses carried out at Rowett Research Institute (1972). † G. H. Francis (1972)—unpublished surveys.

by the dietary treatments, differences in Cd concentration noted in lamb livers 7-8.5 weeks after birth probably arose as a consequence of these lambs ingesting Cd-supplemented diets provided to their dams during lactation.

Although Cu analysis of the livers of one-day-old lambs suggested that treatments providing 7.1 and 12.3 p.p.m. dietary Cd (groups C and D) may have restricted liver Cu storage during foetal development, overall treatment effects were not significant at this stage ( $0.1 > P > 0.05$ ). There was much more restriction of liver Cu storage in lambs aged 7-8.5 weeks. By this time the Cu concentrations in the livers of lambs of groups B and D (3.5 and 12.3 p.p.m. dietary Cd) were only 22 and 13% of those in livers of lambs from ewes on unsupplemented diets. One lamb in group C had a liver Cu concentration of 161 p.p.m. and this was accompanied by a very high liver Fe content. Despite the possibly atypical character of these results they have been included in the calculation of the mean Cu value for this group; nevertheless, the overall effect of Cd supplementation on lamb liver Cu concentration at this stage remains highly significant ( $P < 0.01$ ). There was a similar but lesser effect of Cd on Cu storage in the liver of the ewes ( $P < 0.05$ ).

Although supplementation of the diet with Cd had no effect on liver Zn concentration in the parent ewe, the lambs show trends which suggest an antagonistic effect of Cd on Zn storage.

The kidneys of day-old lambs showed no significant Cd concentrations, further evidence again suggesting that the placenta provides an effective barrier to Cd transfer. There were marked treatment differences both in 7-8.5 week lambs and the ewes. Group mean values for lamb kidney concentrations (p.p.m. d.m.) for groups A to D were  $<0.15$ ,  $6.1 \pm 1.8$ ,  $11.6 \pm 5.7$  and  $45.8 \pm 4.6$  respectively. The corresponding figures for ewe kidney were  $4.8 \pm 2.0$ ,  $7.7 \pm 1.0$ ,  $17.1 \pm 4.1$  and  $30.1 \pm 6.0$ . No significant effects of Cd administration on kidney Cu, Zn or Fe concentrations were detected.

The only clinical manifestation of Cu deficiency in the course of this experiment was a deterioration in wool quality (a loss of "crimp") in ewes receiving 12.1 p.p.m. Cd. The severity and rapidity of the decline in liver Cu status, particularly of lambs, are, however, noteworthy in light of their short period of exposure to Cd. Longer-term studies are in progress. Exposure to dietary Cd concentrations within the range encountered in contaminated pasture has a marked depressive effect on the Cu status of the sheep and particularly of the growing lamb. Cunningham<sup>11</sup> reported a high incidence of bone fragility and some cases of ataxia in lambs having  $<34$  p.p.m. liver Cu. This concentration was, however, exceeded in only 2 of 15 lambs receiving Cd at the end of our experiment. Cadmium supplementation had no influence on the Cu content of brain tissue in our lambs and no "swayback" occurred, possibly because

of the late stage of pregnancy at which Cd administration was initiated. Observations by R. B. Williams (unpublished data) suggest that Cu accumulation within brain tissue occurs primarily during early foetal development.

Work by Goodman and Roberts<sup>1</sup>, the data from our own analyses and those of Francis (unpublished data) suggest that herbage contaminated with Cd of industrial origin also carries abnormally high concentrations of Zn and Cu (Table 3). The concentrations of Zn detected in some herbage samples in these surveys substantially exceed the threshold concentration (200-400 p.p.m.) above which Zn induces clinical effects attributable to Cu deficiency in rats and chicks<sup>2,3</sup>. No similar studies have yet been carried out on ruminants. Whether the high intake of Cd and Zn induces Cu deficiency in animals consuming such herbage depends on the availability of these metals in the forms deposited. Their adverse effects as Cu antagonists may be offset by the high levels of Cu often encountered in such herbage, if this Cu is also in an available form. Seasonal changes in the total Cd, Zn and Cu content of such herbage are great, with low contents in summer and high in winter. The timing of these changes in relation to periods of sensitivity to restrictions in Cu supply during early growth of animals will also have a great bearing on whether high intakes of Cd and Zn produce adverse clinical effects. These aspects require careful investigation before the possible hazards of the situation can be assessed.

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## Female Homosexuality

EISINGER *et al.*<sup>1</sup> conclude from a study of 37 volunteer members of a lesbian organization that, although there were few or no organic differences between lesbians and normal women, on the Eysenck personality inventory the lesbians had significantly higher scores for neuroticism, and lower for extroversion, thus "showing the lesbian group to be clearly dysthymic, that is, prone to anxiety and nervousness, with obsessive tendencies".

It should be emphasized that this conclusion, which has received some press publicity<sup>2</sup>, ought not to be generalized unless it can be shown that willingness to become a member of a "lesbian organization", and voluntarily to submit to such inquiries, is not itself correlated with anxiety, nervousness, and obsessive tendencies. That is far from being self evident: and yet, without proof that the small and self-selected sample really was typical, it would be quite unsafe to apply these results to the population of homosexual women as a whole.

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## GENERAL

### Measurement of Signal-detectability

A NEW measure,  $C$ , of a subject's ability to detect a signal has been postulated<sup>1</sup>; there are, however, a number of shortcomings to this communication.

Hammerton and Altham refer to a signal-detection task in which, at successive cued instants, a stimulus is presented which is either signal superimposed on noise or noise alone. The subject is instructed to rank his response to each stimulus, example responses being:

Yes, the signal is present (given rank 1); and  
No, the signal is not present (given rank  $r$ ),  $r \geq 2$

The  $(r-2)$  remaining categories of response refer to intermediate degrees of certainty. The signal-detection-theory (SDT) analysis of this experiment for  $r > 2$  does not, as asserted by the authors, require that the two assumed underlying distributions<sup>2</sup> have equal variances; for  $r > 2$  the variance ratio is simply introduced as a further parameter and estimated by, for example, the method of maximum-likelihood<sup>3-5</sup>. This procedure allows far more flexibility in the SDT model, though, if the variance ratio is very different from unity, at the same time complicates the measurement of detectability<sup>4,6</sup>.

Further, I have shown<sup>6</sup> that the predictions of the SDT model with underlying uniform (rectangular) distributions agree well with those of the model with underlying normal distributions. Therefore  $d'$ , the difference in mean values of the two populations, would seem to be a robust measure with respect to the form of the underlying distributions.

The most serious shortcoming of the communication is the statement that "It can be shown that  $C$  is monotonically related to  $d'$ ". This statement is misleading as it implies that  $C$  and  $d'$  measure, the same thing; this is not so.

In the simplest case,  $r=2$ , the SDT model requires a variance ratio of unity. Let us suppose that a subject has  $S$  stimuli that are signal and  $S$  stimuli that are noise and that his responses are as follows:

|        | Yes       | No         |
|--------|-----------|------------|
| Signal | $\alpha$  | $S-\alpha$ |
| Noise  | $S-\beta$ | $\beta$    |

The measure  $C$  proposed<sup>1</sup> gives

$$C = \alpha/S - (1 - \beta/S)$$

that is,  $C = \Phi(z - d') - \Phi(z)$

where  $\Phi$  is the standard normal distribution function and  $d'$  and  $z$  are the usual SDT parameters, the measure of the subject's signal-detectability and the cut-off on the "decision axis",  $z$ . We see from this relationship that  $C$  is quite a different measure from  $d'$ . A frequent finding in SDT analyses of vigilance studies<sup>7</sup> has been that over a long period of time a subject's  $d'$  value remains fairly constant but his  $z$  value increases, that is, his power of detectability remains the same but he becomes more cautious as time-on-task progresses. The parameter  $C$  would, in such an example, indicate a change in the subject's power of signal-detectability, quite at variance with the SDT finding. As the success and popularity of SDT are due to its separation of omissions and false-positives into  $d'$  and  $z$ , with just the former as the measure of signal-detectability (a measure that has given "meaningful" results) I find it unlikely that  $C$  will prove to be of any general practical use. More discussion on  $C$  and  $d'$  is given by Altham<sup>8</sup>.

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### Distance between Sets

By proving the triangle inequality, Levandowsky and Winter<sup>1</sup> show that the measure of dissimilarity of two sets

$$d(S_i, S_j) = 1 - \frac{|S_i \cap S_j|}{|S_i \cup S_j|} \quad (\text{absolute value denoting a set measure})$$

can be used as a distance function. The proof given is, however, surprisingly complicated and they ask whether a simple proof exists. Here is one.

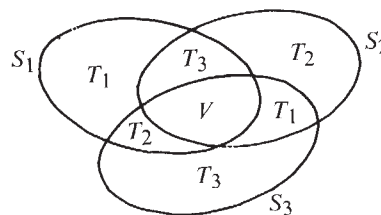


Fig. 1 Representation between sets and sub-sets.

Considering the triangle inequality for the three sets  $S_i$  let  $U = \cup S_i$ ,  $V = \cap S_i$  and  $T_i$  be the three sets shown in Fig. 1.

$$\text{Then } \frac{|T_1| + |T_2| + |T_3|}{|U|} = 1 - \frac{|V|}{|U|} \geq d(S_i, S_j) \geq \frac{|T_i| + |T_j|}{|U|}$$

from which the triangle inequality follows immediately.

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<sup>1</sup> Levandowsky, M., and Winter, D., *Nature*, **234**, 34 (1971).



# BOOK REVIEWS

## Life from Elements

*Origins of Life II. Cosmic Evolution, Abundance and Distribution of Biologically Important Elements.* Edited by Lynn Margulis. (Proceedings of Second Conference on Origins of Life.) Pp. vii+238. (Gordon and Breach: New York and London, 1972.) £12.30. (Published for the Interdisciplinary Communications Program.)

IN 1965 the Interdisciplinary Communications Program planned five annual conferences on the "Origins of Life". In the event, only four were held before funds were cut off: this volume reports the second of these four conferences, held in 1968 in Princeton. The ICP have been trying to relieve one of the bottlenecks of scientific communication. Exponential growth has vitiated the usefulness of both current scientific literature and attendance at international conferences. There is too much literature to read, there are too many conferences to go to, and there are too many people there when you get to them. The ICP have attempted something new; they have organized really small round-table conferences, limited strictly to twenty-five participants. They have encouraged informal, probing conversation, and they have attempted to bring the benefit of the ensuing cut-and-thrust debate to the scientific world at large by publishing a verbatim version. The success or failure of their attempt rests squarely on the shoulders of the editor, who in this case is again Lynn Margulis. This time she has had a more difficult task. Nine of the participants had also attended the first conference (see *Nature*, 235, 404; 1971), but of the sixteen new recruits, two (Edward Anders and Preston Cloud) did not seem to approve of the proceedings; at any rate they insisted that all their contributions should be deleted. This is much to be regretted, for they are both of them prodigious workers, they have been particularly active in the fields under discussion, and they are both outstanding in any controversy. The volume cannot but be poorer for their defection. Nevertheless, there are still parts that make exciting reading.

The first conference introduced a section on "The Fossil Record" by a short but stimulating debate on the evolution of the atmosphere. The present volume is devoted to an expansion of that debate. The composition of the panel was therefore chosen differently. There were more astronomers and physicists, fewer organic chemists. Not only the Earth's atmosphere was considered, but that of other planets, especially Venus and Mars.

The book is divided into three sections. The first deals with cosmic abundances, and the earlier discussion during this section was dominated by H. S. Brown, H. E. Suess, and A. G. W. Cameron. Brown's exposition of the planetary compositional hierarchy was exciting but it did not give rise to a debate; instead, Cameron introduced a particular model for planetary evolution that led to an unprofitable discussion, and terminated with the conclusion that it is highly improbable that life ever did originate. Subsequently others of the team joined in a more general debate, but as presented, with Anders's contributions deleted, it lacks cohesion. This section of the book is not a success.

The second section is devoted to the evolution of the Earth's atmosphere. This again starts badly, with disjointed fragments resulting from the deletion of Cloud's contributions. But then Margulis comes in with a lucid account of the physiological needs of procaryotes and eucaryotes—a potted version of her own book on the *Origin of Eucaryotic Cells*—and she is followed by H. D. Holland, L. G. Sillen, A. E. Ringwood and D. L. Anderson, who each develop models for different controlling parameters. The discussion is summarized by Carl Sagan. This section forms the most satisfactory part of the book. Indeed, if it could be extracted it would make a fine introduction to a course on the evolution of the atmosphere.

In the third section R. M. Goody, S. I. Rasool and L. D. Kaplan talk about the atmosphere of Venus, and B. C. Murray about that of Mars. There is a lively discussion, with most of the team joining in, but (as one might expect) the debate on Venus seems to

have little relevance to the origin of life, and that on Mars is too inconclusive to excite much interest.

This second conference was meant to make good the deficiencies that became evident during the first. The book is worth reading, but it does not really succeed in its object. Some of the ideas propounded in the first volume may have been wild, but at least they were fun. Heavy documentation does not mix well with off-the-cuff discussion. This book is too much of a compromise.

P. C. SYLVESTER-BRADLEY

## Powell's Papers

*Selected Papers of Cecil Frank Powell, Nobel Laureate, FRS.* Edited by E. H. S. Burhop, W. O. Lock and M. G. K. Menon. With a tribute by Victor F. Weisskopf. Pp. xiii+455. (North-Holland: Amsterdam and London, 1972.) Dfl. 80; \$23.50.

CECIL POWELL, who died suddenly a short time after his retirement from the University of Bristol in 1969, was known to his friends and colleagues in many roles. There was the pioneer of early research on elementary particles, the leader of later research programmes in the same field, the tireless committee chairman and pillar of the university, the senior scientist increasingly concerned with major world problems and using his influence in the search for their solutions, and there was the storyteller with his unrivalled tales of early life.

In this volume we have a collection of papers reflecting these varied roles, presented in accordance with Powell's own high standards of literary and technical perfection. A tribute by V. F. Weisskopf, former Director-General of CERN, is followed by the unfinished autobiography on which Powell was working as retirement approached. The later chapters are reprints of published papers, on his early work in Cambridge and Bristol, especially on the mobility of ions in gases, on the development in Bristol of the photographic emulsion technique for the study of elementary particles, and on the successful exploita-

tion of this technique in the discovery of mesons and hyperons; this work, which formed the foundation of modern particle physics, led to the award of the Nobel Prize for Physics in 1950. A final chapter, "Science and Society", is made up of reprints of speeches and papers on international and social problems, and the place of science in the modern world.

To those of us who knew Cecil Powell, and still appreciate the influence which he had on our lives, this book comes as a welcome reminder of the qualities on which his influence was founded. To those who did not know him personally, it will bring to life a period in which physics underwent great changes, and scientists came to see that they carried special responsibilities.

As a contribution to the history of science, it will present to the future student a factual and human record of an important period in the development of physics and of scientific attitudes. One is tempted to wish for a more restricted selection of reprints of papers which have already been published elsewhere; but it would have been difficult to reduce the number of reprints without obscuring the important point that our present level of understanding has been reached through a process of development in which each step has required meticulous examination and careful formulation.

A longer autobiographical section would of course have been welcome, but the editors can hardly be held responsible for the fact that it was not available. They are to be congratulated on having produced a very appropriate record of a memorable life.

W. M. GIBSON

## Essays on Fossils

*Studies in Vertebrate Evolution.* Edited by K. A. Joysey and T. S. Kemp. Pp. 284. (Oliver and Boyd: Edinburgh, June 1972.) £7.

IN the first of this collection of essays, presented to Dr F. R. Parrington, FRS, on his retirement, Dr Whiting has interpreted the cranial anatomy of the ostracoderms in terms of the larval lamprey. He follows Lindstrom in postulating that the most anterior nerve visible in the cephalaspids is the trigeminal ( $V_{3,3}$ ). This makes the arrangement of the nerves in cephalaspids like that in the ammocete. He is too cautious to commit himself to the re-interpretation of the branchial pouches, but these, I suspect, may eventually also turn out to be rather more like those in the ammocete than appeared at first sight.

In other contributions, Dr Shute analyses the elements which make up the vertebrae; Dr Andrews reinterprets the "*Eogyrinus*" material in the Hancock Museum, Newcastle; and a new labyrinthodont from Queensland is described by Dr Howie.

Dr Panchen writes on the interrelationships of the earliest tetrapods, postulating that the prototetrapod was a comparatively small animal. I am bothered by this, because I think that the greatest advantage of locomotion on land would be to a large aquatic carnivore. Being able to move from one body of fresh water to another, this prototetrapod could increase his "catchment area" and therefore his food supply and hence grow to a larger size than a form confined entirely to a single body of water. Crocodiles occupy this niche at the moment, and I assume that the ichthyostegids, which were amongst the largest predators of their time, occupied the same niche in the Upper Devonian.

Dr Cruickshank's paper on proterosuchian thecodonts, the most primitive archosaurs and the starting point of archosaurian phylogeny, is particularly welcome for fulfilling the need for further information on this group.

There is a long and largely theoretical paper on the archosaurian pelvis and hind limb by Dr Charig. I agree with his thesis that all archosaurs have not passed through a bipedal stage in their ancestry; I regret that he has held to the old-fashioned idea that the theropodous dinosaurs held their bodies in an upright posture during locomotion. Newman's analogy with birds is probably correct and certainly leads to a more plausible-looking reconstruction. If the horizontal position of the skeleton of *Tyrannosaurus* in the British Museum (Natural History) is due to the limitation of space, this was a fortunate accident. Professor Bellairs has written on snakes and Dr Cox describes a new fossorial dicynodont. The Bauriomorpha, like the Proterosuchia, have figured largely in the discussion of phylogenies despite our ignorance of many details of their anatomy. Mme Mendrez's description of a new bauriomorph, *Regisaurus jacobi*, has added to our knowledge of this group. Dr Kemp, who has been carrying on the traditions of Dr Parrington at Cambridge, gives us an account of the jaw articulation and musculature of the whaitsid Theracephalia. From Dr Crompton we have an account of the cynodont jaw articulation. He describes a surangular-squamosal jaw articulation developing in cynodonts to supplement the normal reptilian articulation (articular-quadrates). The former he considers the precursor of the mammalian dentary-squamosal articulation.

The classification of the insectivores

is discussed by Professor Butler, and Dr Joysey writes on the fossil species in space and time.

The final paper is by Professor Kühne on progress in biological evolution. Like the editors, I should prefer him to have written on Jurassic mammals because it is in a very real sense his own subject. The great revival of interest in early mammals, and the fruitful work of the last twenty years by many workers, stems from Professor Kühne more than any other man.

The book is well illustrated.

K. A. KERMACK

## Cyanocobalamin

*Inorganic Chemistry of Vitamin B<sub>12</sub>.* By J. M. Pratt. Pp. ix+347. (Academic: London and New York, May 1972.) £6; \$18.75.

Vitamin B<sub>12</sub>, or cyanocobalamin, was isolated in 1948; it is a diamagnetic, six-coordinate cobalt(III) complex, and the study of B<sub>12</sub> has attracted an extremely wide range of investigators—in medicine, agriculture, microbiology, biochemistry, organic chemistry, and coordination chemistry. In 1821 the disease known as pernicious anaemia was described, but this disease remained incurable until the "anti-pernicious anaemia factor" was discovered in raw liver in 1934. After the isolation of the factor in 1948 the next main development was the large-scale production of B<sub>12</sub> by fermentation techniques in the early 1950s. The next decade saw a flurry of activity in investigating corrinoids, culminating in 1958 with the isolation of the so-called "coenzyme" form of a corrinoid. Hodgkin showed that the coenzyme possessed an organo-ligand. This book is about the role that inorganic coordination chemists have played in investigating B<sub>12</sub>. It is written by one who, in collaboration with R. J. P. Williams in Oxford, has contributed a great deal to our present knowledge.

This volume is extremely well put together and includes a helpful chapter on nomenclature of corrinoid systems and a brief chapter on the natural occurrence of B<sub>12</sub> in biological systems. As well as being implicated in the synthesis of methionine, B<sub>12</sub> has recently been shown to be capable of producing dimethylmercury from other mercury compounds, a topical, albeit sinister, reaction. But the book is mainly concerned with the inorganic aspects of recent research and Pratt details this thoroughly. The inorganic chemist has gained much from the work described, for many cherished notions have been set aside, viz., that cobalt(III) com-



pounds are kinetically inert (the corrinoids are not); that a compound containing a metal-alkyl bond is extremely unstable (the methyl-corrinoids are quite remarkably stable towards acid, alkali, and heat, although they are photosensitive); that cobalt(III) complexes are six-coordinate (many of the organo-cobalt(III) complexes are penta-coordinate); and that the properties of metal complexes are influenced by crystal field effects (no crystal field effects are observed with  $B_{12}$  derivatives).

Because corrinoids contain stable Co-alkyl bonds (they are the only examples of stable naturally occurring organometallics) they have contributed significantly to studies of  $\sigma$ -bonded organoligands, in contrast to the more commonly studied  $\pi$ -bonded ligands. Similarly, the photochemistry of metal complexes, initiated by internal transitions on the ligand, has been further developed by recent work on  $B_{12}$ .

This book would seem to represent required reading for anyone professionally interested in bioinorganic chemistry, but biochemists and coordination chemists generally would gain much from it. It is very readable and the chapters liberally cross-referenced. At £6.00 it is good value for money.

C. A. MCAULIFFE

## Evolution of Nutrition

*Biology of Nutrition: The Evolution and Nature of Living Systems: The Organization and Nutritional Methods of Life Forms.* Edited by Richard N. T. W. Fiennes. Pp. xv+681. (Pergamon: Oxford, February 1972.) £18.

THIS book of almost 700 pages endeavours to combine a textbook of biochemistry, a textbook of ecology, a treatise on the origin of life (emphasizing that the protocells, borrowing organic molecules from the medium, represent the first forms of nutrition), a treatise on bioenergetics, and a treatise on biochemical evolution, these additional texts being provided as the accessory knowledge necessary for the perusal of the textbook on nutrition in the context of evolution, which the reader is expected to untangle from this skein.

The evolution of nutrition and of chemical needs depends, on the one hand, on the evolution of ATP-generating processes, and on the other hand, on losses of biosynthetic capacities. These aspects of molecular evolution do not appear clearly defined or placed in order in the book as one would wish, and the distribution of the material remains traditional, placed along the taxa of organisms. Perusing the table of contents, the reader will be able to identify data on a number of nutritional sys-

tems: bacteria and micro-fungi, viruses, protozoa, fungi, lichens, marine algae, pelagic invertebrates, worms, echinoderms, coelenterata, molluscs, tunicates, crustacea, insecta, fishes, amphibia, reptiles, birds . . . indeed, all living creatures with the exception of man.

Little attention is given to the mechanisms of molecular evolution, and to the variations of metabolic pathways along phylogeny, to account for chemical needs. A consideration of the role of ecomones in the establishment of relations between organisms in the networks of the biochemical continuum is also lacking.

In our present concepts, the appearance in history of the different ATP-generating processes may be considered as having taken place in the order (1) fermentation, leading to (2) photo-organotrophy, leading to (3) photolithotrophy, leading to (4) phytotrophy (plant photosynthesis), leading to (5) oxidative phosphorylation (respiration). By using different parts of the book, the reader will be able to collect information on these successive steps or at least on some of them.

The book is divided into five sections among which the monographic treatments of taxonomic groups are more or less clearly placed in order. Section I is devoted to the evolution of the Earth and to prebiotic chemical evolution and in this section are also included a number of chapters on bacteria, viruses and protozoa, defined as "the earliest life forms existing today".

Section II deals with the acquisition of free energy and its relation to nutrition and growth, while Section III is concerned with the evolution of the habitat. In Section IV emphasis is placed on the living communities and ecological and ethological aspects.

The general leading idea of the book seems to be focused on the stages from the trappings of energy to the nutritional systems of the life of higher organisms. Many of these stages still persist on the Earth and they are necessary for the continuation of man's life. The popular appeal of the concept of pollution is thereby introduced into the book.

As stated by the editor, the volume was originally planned to deal with the nutrition of lower organisms, by which he understands all forms of life except man and domestic animals, whose nutrition is already dealt with in another volume of the same series. The patient reader will be able to extract from the mass of ornaments with which it is overburdened an original book on the nutrition of "lower organisms" written by competent experts. Contrary to the fears of the editor, this comparative treatment of nutrition is not "unregarding" nor "dull" nor "prosy", but, on the contrary, very useful.

MARCEL FLORKIN

## Scientific Teaching

*Instructional Science: An International Journal.* Editorial policy board: G. F. Brieske, B. N. Lewis, M. MacDonald-Ross and R. Wm. Smith. Vol. 1, No. 1. Pp. 151. (Elsevier: Amsterdam, March 1972.) Institutional subscribers, Dfl. 87.50, \$27.30, £10.60. Personal subscriptions, Dfl. 45.50, \$14.20, £5.50.

*Instructional Science* is a new journal in the general area of educational technology. Its declared aim is to publish material relating to teaching, training or communication in general, that is, "with the whole range of processes and considerations which can influence, either directly or indirectly, the impact and effectiveness of attempts to instruct". These are said to range from teaching in the nursery or the classroom to the kinds of instruction attempted by politicians, lawyers, churchmen and the mass media.

The editorial board has wisely maintained a practical slant by using the word "instruction" rather than "learning" in the title. They are obviously aware of the necessity to avoid becoming immersed in material bearing on the learning process itself. We are told that "certain kinds of manuscript on the subject of learning—experiments with rats and pigeons, abstract excursions into automata theory, investigations into the biological bases of memory"—may be refused.

The first article by Professor G. P. Meredith consists of a long, personal and interesting review of its author's work and ideas on epistemics. It is fitting that a new journal should permit itself the luxury of publishing this type of material on rare occasions, but it sets a high standard for what is to follow, and the second article, "Perception of the Future and the Future of Perception", might best have been omitted. It also is discursive, but technically inaccurate, and as a medium of instruction difficult to comprehend.

The remaining three articles are (1) "Style and Effectiveness in Education and Training", by Ivor K. Davies; (2) "Reflections on the Process of Visual Design", by Nicholas Tomlinson and John Stevens; and (3) "Computer Frequency Control of Vocabulary in Language Learning Reading Materials", by D. Barton Johnson. These are of a high standard. Book reviews and correspondence are also included.

*Instructional Science*, besides acting as a general forum for those interested in the technical aspects of instruction, will be of use to those involved with instruction of one form or another, but who have difficulty isolating useful material bearing directly on this topic from the mass of articles published in the journals.

C. E. M. HANSEL

## Caledonides

*Scandinavian Caledonides.* By T. Strand and O. Kulling. Pp. x+302. (Wiley: London and New York, April 1972.) £18.75.

THIS long awaited book is not a single or coordinated account of the Scandinavian Caledonides but two more or less completely separate ones for Norway and Sweden. Both are rather old, having been mostly written some seven years ago. The book contains no single map on any scale of the whole Scandinavian Caledonides. The Norwegian section which contains no single map of Norway of any kind directs attention to the one to one million map of Norway (approximately £2 more). The Swedish section contains an outline map of the Caledonian geology of Sweden at a scale of some one to three million but does not fix the map relative to the geographies of either Sweden or Norway. No reference is made to the one to one million map of Sweden, although direct reference is made to the accompanying description. Regional maps are few and the use of tables to organize data and allow easy comparison too infrequent. Kulling's Swedish Caledonian has more the flavour of a research monograph than Strand's for Norway. This is natural because the Swedish Caledonides samples only the eastern part of the mountain system while Strand has to cope with all aspects. Because of the difference, however, and possibly because of the number of geologists working on the Norwegian side in the past decade, Strand's account looks more dated than Kulling's. Both sections of the book lack definition or discussion of the large scale problems the chain offers: perhaps this is editorial policy. While the book has considerable value to anyone interested in the Scandinavian Caledonides, such obvious deficiencies in a member of a series directed at an international audience and above all so magnificently begun by Gansser's book on the Himalayas must be regretted.

R. NICHOLSON

## Cold Mutton

*Atlas of Protein Spectra in the Ultraviolet and Visible Regions.* Edited by Donald M. Kirschenbaum. Pp. xii+290. (Adam Hilger: London; Plenum Press: New York; 1972.) £10.60.

IT is difficult to be kind about this book. In the 1972 Misconceived Enterprise Stakes (which could be run each year for the benefit of scientific publishers, a number of whom would regularly pro-

vide strong contenders) it should be a firm favourite. Most people with experience in ultraviolet and visible spectrophotometry have learnt, often to their cost, that even on one identical sample a spectrum from one laboratory is not going to look the same as the spectrum from another unless very careful control of procedure is maintained. For this reason unselective collections of spectra (like this book) taken from the literature are practically valueless. It might almost be said that the value of any collection of spectra varies inversely with the number of contributors; certainly the useful collections are all of spectra measured under controlled conditions (in intention at least) by a limited number of contributors, and most of these would have been better with fewer contributors and hence greater control. The field of protein spectroscopy is, too, a quite peculiarly hopeless one in which to jettison one's critical faculties, and it seems that, from the attempt made to disarm criticism in the preface, the editor knows this perfectly well.

This may seem a negatively purist attitude; for if, as the editor claims, all the relevant data published in the original paper had been included with each spectrum, it would then have been possible, for the experienced reader at least, to make a personal assessment of its value. But unfortunately in the protein field the relevant data must include much, for example, the preparation and purification of the sample, which is only available (if it is available at all) by reference to the original. There is therefore no basis for making, as the editor suggests, comparisons of subtle differences between the spectra of uncomplexed proteins which occupy much of this book. It would have been more useful, and much cheaper, simply to have listed  $\epsilon$  (1 per cent, 1 cm) values with each of these references, and since in many cases there are no data to calculate this essential parameter the saving in space would be greater still.

So we have a decidedly expensive bibliography of papers on protein work which include spectrophotometric data. Nearly half of the 534 references are for 1966-9, but they go back to 1925, with 32 of them pre-1939. They are listed in alphabetical order of protein names, with a general index, a sources index, and an index of miscellaneous phenomena (effects of pH, irradiation and so on). An index listing proteins according to their prosthetic groups or metal content would have been at least equally useful, because it would then be possible to compare the spectra of, for example, the flavo-proteins, or the copper-containing proteins. It is sad to see the distinguished name of Adam Hilger on a book such as this.

E. A. JOHNSON

## Membraneosophy

*Membrane Structure.* By D. Branton and D. W. Deamer. Pp. 70+24 figures. (Springer-Verlag: Vienna and New York, 1972.) US \$13.10.

So much work is being done on natural membranes, with some progress, that reviewing it seems attractive. (Indeed, one wonders if a shortage of research funds might be accommodated by half the scientific community reviewing work done by the other half. One could draw lots for three-year stints in the lab. or else in the library.) A review on membrane structure has recently been published in the series of monographs with the 18-letter title *Protoplasmatologia*. The series began as a handbook for cell biology; but having reached 9,400 pages, the publishers abandoned this format.

Having set their sights on finding the structures underlying fascinating membrane functions, Branton and Deamer begin with a necessarily short review of present knowledge and then consider in turn protein-lipid-protein (PLP), lipid-protein-lipid (LPL) and particulate models. There is a section on differentiation and specialization, and the authors conclude with a summing up for a modified PLP model since it alone "can reasonably account for the X-ray diffraction results with a variety of membranes, as well as detailed profiles of myelin", and since the model is supported by electron microscopy.

In attempting to amend the PLP model, the authors consider examples of protein hydrophobically bound to a lipid bilayer "without requiring the entire protein molecule to be located within the structure of the membrane". One of these is a structure in which "some of the membrane phospholipids would have one fatty acid chain extended into the membrane interior and the other fatty chain extended towards the membrane surface, where it would interact with hydrophobic binding sites of the surface protein". Broadening the Danielli-Davson proposal of 1935 to this extent is found to be necessary because the PLP concept has some obvious weaknesses.

The book summarizes a broad range of studies: electron microscopy, X-ray diffraction, thermal analysis, electron spin resonance, infrared, optical rotary dispersion, circular dichroism, nuclear magnetic resonance and enzymic hydrolysis of lipids. Because of the volume of work done, it is a difficult field in which to publish a timely and accurate review. Hence one can excuse the unaccountable statement that "there is certainly no good evidence for the strong lipid-protein interactions envisaged by both the PLP and the subunit models": PLP may be a misprint for LPL. A. E. BLAUROCK



# CORRESPONDENCE

## "Drivel" on Katchalsky

SIR,—It is disheartening and distressing to read the outright drivel which has been appearing in both *Nature* and *Science* regarding the murder of Professor Aharon Katchalsky Katzir (for example, *Nature*, **238**, 361; 1972).

Virtually the entire population of the United States now regards the Vietnam war as a horrible blunder, and many of us have held that view since the war began. It is true that in the course of that war innocent civilians have been killed. However, the murder of civilians is not and never has been our Government's war policy. American soldiers are not ordered to kill civilians, and whenever they do so it is either a sick aberration or the result of a horrible error—all in the context of a military conflict.

To equate this wartime killing of civilians by error with the carefully planned, purposeful, braggartly, and wanton murder of random civilians by demented, true-believing zealots, certainly is the high point in sloppy thinking for these troubled times.

Yours faithfully,

WILLIAM A. PRYOR

Department of Chemistry,  
Baton Rouge, Louisiana 70803

## Scientists and Politics

SIR,—A recent editorial in *Nature* (**238**, 61; 1972) described in graphic detail the insulting treatment of Dr Zhores Medvedev at the recent International Congress on Gerontology in Kiev. The same issue of *Nature* also contains correspondence from Prof. John Ziman pointing out other nefarious practices indulged in by the Soviet Government with respect to their scientists travelling

abroad. These items are more especially newsworthy in the light of the observation that "in the past few years, the Russian authorities have been eager to encourage the use of Moscow and other Russian cities for international scientific conferences . . .". Further testimony to the hypocritical attitude of the Soviet bureaucracy towards international scientific exchange comes from the most recent IV International Biophysical Society Meeting in Moscow which seemed plagued with further examples of political interference.

I am a scientist who has occupied resident alien status in the USA for the past 7 years. Since my naturalization proceedings have not yet been fully completed, I reluctantly still carry a South African passport.

During the months preceding the meeting I faithfully complied with all deadlines for submission of abstracts, clearing of Intourist, and application for a visa to enter the Soviet Union. One week prior to my scheduled departure, the all-too-familiar rhetoric emanating from the Soviet Embassy in Washington led me to seek the assistance of a variety of official sources, including the US Office of the Foreign Secretary, the Soviet Academy of Sciences, the International Union of Pure and Applied Physics, and the Organizing Committee of the meeting. Despite the urgent and, to my knowledge, sincere efforts of these bodies, I was not issued the necessary visa. While the fact that I hold a South African passport might not be viewed with much sympathy in many quarters, this does not seem to me germane to the central question at large—denial by any government of any scientist's right to participate in an international scientific meeting anywhere in the world is a flagrant violation of one of the most fundamental tenets of scientific free-

dom and deserves the unqualified condemnation of that government's action.

Although my information at this point is second-hand, my enquiries during the days immediately preceding the meeting led me to understand that other scientists wishing to attend this meeting suffered a similar fate. I am thus forced to the sad conclusion that the time has not yet come when the Soviet Union can extend its scientific hospitality with candour and conviction, and I would emphatically echo the sentiment expressed in *Nature* that "scientific societies in the west should now seriously consider whether they should continue to participate in plans for holding conferences in the Soviet Union. . . ."

Yours faithfully,

ERROL C. FRIEDBERG

Department of Pathology,  
Stanford University Medical Center,  
Stanford, California 94305

## Action at a Distance

SIR,—Is it not rather unfair to call action at a distance a mediaeval notion (in *News and Views*: "Elementary Particles and Cosmology", *Nature*, **238**, 69; 1972)? Surely the Schoolmen, who spoke for the Middle Ages on philosophical matters, denied that any such thing happens.

On the other hand, the notion that the future may have some influence on events, which may seem so strange today, would not have surprised the Schoolmen; for they believed in teleology.

Yours faithfully,

H. L. ARMSTRONG

Department of Physics,  
Queen's University,  
Kingston, Ontario

# Obituary

## Sir Gavin de Beer

SIR GAVIN DE BEER, who died suddenly on June 21, 1972, was a zoologist in the great tradition of Ray Lankester and Goodrich; essentially a comparative anatomist and embryologist, always evolutionary in outlook. His research was rigorous, never trivial in aim but adjusted to clarify important issues. His writing was scholarly and lucid,

mainly addressed, in its scientific aspects, to specialists or to undergraduates reading for honours at a university. He was, however, author of one of the best elementary textbooks on vertebrate zoology (Sidgwick and Jackson, 1928) ever written.

Gavin Rylands de Beer was born in 1899. He married Cicely Glynn, daughter of the Reverend Sir Hubert Medleycott, Bt, who survives him. He

was educated at the Ecole Pascal, Paris, at Harrow and at Magdalen College, Oxford, where he was a Demy, and served as a Lieutenant in the Grenadier Guards in 1918-19.

On taking his final examination he was at once elected Fellow of Merton College, subsequently becoming Sub-Warden, and Demonstrator in the Department of Zoology at Oxford, where he was Jenkinson Lecturer in Embryo-



logy from 1926-38. From 1939-45 he served as Lt-Colonel in the Grenadier Guards, GSO Psychological Warfare. At the end of the war he became Professor of Embryology at University College, London, a position which he held until he was appointed Director of the British Museum (Natural History) in 1950. He resigned in 1960 and became a director of Thomas Nelson, Publishers. In 1967 he settled in Switzerland, whence at the time of his death he had just returned to live in England.

Among his honours may be mentioned Knight (1954), Fellow (and Darwin Medallist) of the Royal Society, DSc (Oxon), Hon. ScD (Cantab), Hon. D-ès-L (Lausanne), Hon. D de l'Univ (Bordeaux), Chev Lég d'Hon.

One of the most remarkable features of de Beer's work is the unfailing high standard of scholarship that he maintained in spite of the diversity and scale of his output. He wrote 16 books and 110 articles on zoology, 5 books and 40 articles on the history of science, 9 books and 52 articles of a biographical character, 9 books and 80 articles on Switzerland, 3 books and 12 articles on military affairs, modern and ancient, and 3 books and 18 articles on a series of varied topics. The industry and learning involved seem overwhelming, especially in a man who found time for wide artistic and general interests; he was a Trustee of the National Portrait Gallery and a Fellow of the Society of Antiquaries.

De Beer's researches on comparative anatomy and embryology were particularly concerned with the vertebrate head, especially its segmentation. To this subject he made notable contributions. They were much assisted by his method of producing large-scale models in plaster of Paris, each built up from a sequence of microscope slides. His collection of these reconstructions, representing different stages in development, not only threw light upon fundamental vertebrate anatomy but pro-

vided teaching material of great value. In particular, they demonstrate the developmental stages from the chondrocranium to the bony skull. In carrying out that work he especially studied a number of sharks, primitive and specialized bony fish and the shrew. Related to the same subject was his investigation of the pituitary from the lamprey upwards, upon which is based the evolutionary sequence of that organ as accepted today.

More difficult to evaluate in its contribution to modern zoology was the rise of experimental embryology under the leadership of Ross Harrison and Hans Spemann, so fully described by de Beer in books and articles. It may be hard to realize how greatly that subject dominated zoological thought in the 1920s. Those establishing it then may perhaps have laid a foundation upon which the superstructure is in the main yet to be built.

During the decades when he was Director of the Natural History Museum, although deeply involved in administration, de Beer was bringing together the materials for his great *Atlas of Evolution* (Nelson, London). This, however, was not published until 1964.

Though comparative anatomy and embryology were the principal subjects of his research, he read widely and was keenly interested in all aspects of zoology and its history. In particular, he was fascinated by the work and writings of Darwin, upon which he threw much light; and he produced a reprint of the sixth edition of the *Origin of the Species*, the last to appear during its author's lifetime, to which he added a discriminating preface. He hailed the experimental study of evolution in wild populations, a subject developed during the last forty years or so, as the fulfilment of Darwin's hopes.

De Beer was essentially a man of culture; and he who was himself a distinguished scientist looked with no favour upon those whose interest is limited to science. Indeed, he personified a type more frequent in his youth, when the aim of education was to educate rather than to qualify candidates to pass examinations.

Among his many biographical, historical and other writings, those dealing with Hannibal's march into Italy have aroused special interest. They seem to have solved the age-old problem of the route by which the general brought his troops and his elephants across the Alps.

No assessment, however brief, of de Beer's life and character should omit reference to his outstanding ability as a linguist. He spoke German almost perfectly, his Italian was fluent and polished and, without raising a doubt,

he could pass as a Frenchman in France.

Casual acquaintances tended to find his encyclopaedic knowledge poured out on the widest variety of topics rather daunting in conversation, unless they realized that his interest in their remarks, if worthwhile, could be as keen as in anything he was saying himself. Those who knew him well discovered that in his friendship they possessed something enduring, for he was always to be relied upon, always the same.

## Announcements

### University News

**Professor A. C. Turnbull**, Welsh National School of Medicine, has been appointed Nuffield professor of obstetrics and gynaecology in the **University of Oxford**.

### Appointments

**Mr W. B. S. Walker** has been appointed a part-time member of the **UK Atomic Energy Authority**.

### Miscellaneous

The 1971 **Kalinga prize** for the popularization of science has been awarded to **Professor Pierre Auger**.

The Polish government has awarded the Knights' Cross of the Order of *Polonia Restituta* to two Soviet biologists, **Academicians Pavel P. Luk'yanenko** and **Vasilii N. Remeslo**, for their work in developing the high-yielding strains of wheat 'Mironovskaya-808', 'Avrora' and 'Kavkaz', which together now account for some 25% (by area) of all Polish wheat growing.

The **Ramsay Memorial Fellowships** trustees have made the following awards of new fellowships for the year 1972-1973: a general (British) fellowship to Dr P. J. Derrick at University College London; a Glasgow fellowship to Dr D. N. J. White at the University of Glasgow; a Canadian fellowship to P. J. Young at the Davy Faraday Research Laboratory of the Royal Institution; two Netherlands fellowships to Mr Yoe Han Thoeng at Royal Holloway College, University of London, and Dr J. F. M. Aarts at University College London, respectively; a New Zealand fellowship to Mr Charles R. Clark at the University of Stirling; a Spanish fellowship to Dr R. M. Utrilla at the Davy Faraday Research Laboratory of the Royal Institution. The trustees have renewed the fellowships of Dr F. M. Benoit, Dr P. Chamberlain, Mr B. L. Dickson, Dr S. Uemura and Dr L. V. Woodcock for 1972-73.